Herbivore-induced indirect plant defences of Lima bean (*Phaseolus lunatus*, Fabaceae)

Dissertation
zur Erlangung des akademischen Grades
doctor rerum naturalium (Dr. rer. nat.)

vorgelegt dem Rat der Biologisch-Pharmazeutischen Fakultät
der Friedrich-Schiller-Universität Jena

von Diplom-Biologe
Christian Kost

geboren am 9. September 1975 in Bad Sobernheim

Gutachter

1. Prof. Dr. Martin Heil (Universität Duisburg-Essen, Deutschland)

2. Prof. Dr. Wolfgang W. Weisser (Friedrich-Schiller-Universität Jena, Deutschland)

3. Prof. Dr. Paulo S. M. C. de Oliveira (Universidade Estadual de Campinas, Brasilien)

Tag der Doktorprüfung: 30.01.2006

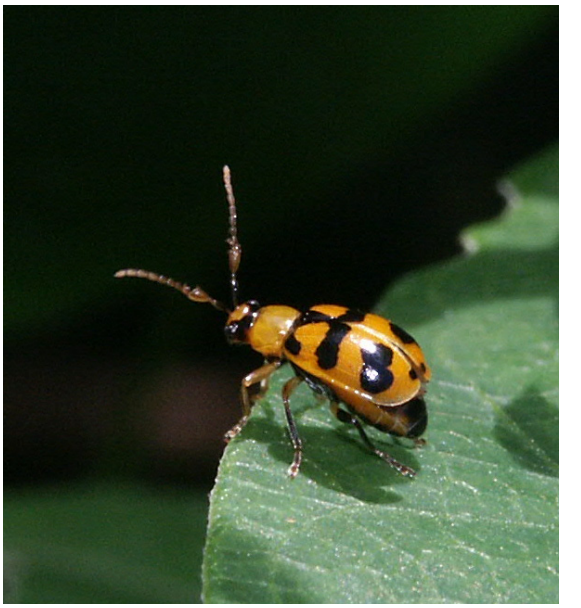
Tag der öffentlichen Verteidigung: 21.04.2006

Preface

This thesis is the end of a journey that lasted almost three years and that took me along the way twice to Mexico. When I look back and say that I learned a lot during this time, I mean not only scientific skills and knowledge that I acquired in the course of this interesting, inter-disciplinary project, but I also appreciate invaluable experiences that I gained during my stays abroad and my work in Jena.

An enterprise like this Ph.D. project can certainly not be accomplished without the help of numerous people that contributed in numerous ways either directly or indirectly to its success. To these people, that crossed my path during the past three years and made this time unforgettable, I would like to express my warm gratitude.

First of all, I wish to thank my doctoral advisor **Prof. Martin Heil** (University Duisburg-Essen) for the opportunity to work on this exciting project in Jena and Mexico, his invaluable support, and stimulating discussions throughout all stages of this thesis. His advice and encouragement were an added spur to my ambition.



Just as much I owe thanks to **Prof. Wilhelm Boland** (MPI for Chemical Ecology) for teaching organic chemistry to an ecologist, for always having an open door, his contagious enthusiasm, stimulating discussions and his unrestricted support.

Beyond I am indebted to **Prof. Wolfgang W. Weisser** (FSU Jena) for kindly accepting the official supervision as a representative of the FSU Jena and giving me the opportunity to present and discuss my results in his group's seminar.

To **Silvia Schmidt** I am endlessly grateful for her love, company during all the years, lots of encouraging words when things were tough and giving me the feeling of not being alone.

I owe substantial debt to **Sven Adolph** and **Mesmin Mekem Sonwa** who patiently taught me organic synthesis and always kept an eye on me. Also **Stefan Bartram**, **Christoph Beckmann**, **An-**

Photograph: *Cerotoma ruficornis* OLIVIER on a leaf of *Phaseolus lunatus*. Source: CHRISTIAN KOST.

dreas Habel, **Georg Pohnert**, **Doreen Schachtschabel** and **Dieter Spiteller** always stood by me with moral and practical support.

I am especially grateful to **Anja Biedermann** for introducing me into various methodological techniques and assisting me with laboratory work. Furthermore, I am thankful to **Angelika Berg** for taking considerable care for the Mexican bean beetles. A great deal of credit goes to **Udo Kornmesser**, **Andreas Weber**, **Tamara Krügel**, and the rest of the **greenhouse team** for raising countless plants.

For there company, help and a great time in Mexico I thank **Kerstin Ploss**, **Martin Heil** and **Ralf Krüger**. I am especially grateful to **Perla Monica Martinez Cruz** (Universidad del Mar, Puerto Escondido, Mexico) for a warm welcome in Mexico as well as logistic support in the field. **Janine Rattke** (Elsie Widdowson Laboratory, Cambridge) is thanked for helping with the GC-MS analysis of nectar samples and **Manfred Verhaagh** (Staatliches Museum für Naturkunde, Karlsruhe) for the identification of collected ants. Further, I want to express my thanks to **Birgit Schulze** for introducing me into the secrets of JA-extraction and lots of inspiring discussions.

Several people benefited this thesis by discussions or reading drafts of manuscripts and commenting on them. Their advice was time-consuming to them but immensely valuable to me: **Martin Heil** (University of Duisburg-Essen), **Paulo S. Oliveira** (Universidade Estadual de Campinas, Brazil), **Victor Rico-Gray** (Instituto de Ecología, Veracruz, Mexico), **Flavio Roces** (University of Würzburg), **Georg Pohnert** (Ecole polytechnique fédérale de Lausanne, Switzerland), **Wolfgang W. Weisser** (Friedrich-Schiller-University Jena), **Ian T. Baldwin**, **Wilhelm Boland**, **Jesse Colangelo-Lillis**, **Rüdiger Dietrich**, **Maritta Kuhnert**, **Heiko Maischak**, **Axel Mithöfer**, **Birgit Schulze**, **Silvia Schmidt**, **Thomas Wichard** (all Max Planck Institute of Chemical Ecology, Jena) as well as a number of anonymous referees.

I especially enjoyed lively scientific and non-scientific discussions as well as tantalising games evenings with my coffee-round **Lars Clement** and **Heiko Maischak** as well as with my group-spanning interest and discussion group **Markus Benderoth**, **Caroline C. von Dahl**, **Markus Hartl**, **Anja Paschold** and **Silvia Schmidt**.

Finally I would like to thank my family for their love, support, encouragement and the privilege to realize my dream of becoming a natural scientist.

Financial support by the **German Research Foundation** (DFG Grant He 3169/2-1, 2, 3, 4) and the **Max Planck Society** is gratefully acknowledged.

This work would not have been possible without your help!

Contents

Abbreviations, acronyms and symbols

ACC	1-aminocyclopropane-1-carboxylic acid
A_{IS}	peak area of the internal standard (1-bromodecane)
AFLP	amplified fragment length polymorphism
AOC	allene oxide cyclase
AOS	allene oxide cyclase
ALA	alamethicin
A_{VOC}	peak area of a volatile organic compound
CAM	calmodulin
CDPK	calcium-dependent protein kinase
CLSM	closed-loop-stripping method
DDQ	4,5-Dichloro-3,6-dioxo-1,4-cyclohexadiene-1,2-dicarbonitrile
DMNT	(3*E*)-4,8-dimethylnona-1,3,7-triene (C_{11}-homoterpene)
DW	dry weight
EAG	electroantennogram
EF	extrafloral
EFN	extrafloral nectar
EI-MS	electron impact mass spectrometer
EI-MS	electron impact high resolution mass spectrometer
ERF	ethylene-responsive transcription factor
ET	ethylene
eq	equivalent
FAC	fatty acid–amino acid conjugate
FAD	ω-3 fatty acid desaturase
Fig.	figure
FPP	farnesyl diphosphate
GC	gas chromatography
GC-MS	gas chromatograph coupled to a mass spectrometer
GPP	geranyl diphosphate
HI	herbivore-induced
HI-VOCs	Herbivore-induced volatile organic compounds
HIPV	herbivore-induced plant volatile
13-HPOT	13(*S*)-hydroperoxy-9(*Z*),11(*E*),15(*Z*)-octa-decanoid acid
HPL	13(*S*)-hydroperoxy-hydroperoxid lyase
HRMS	high resolution mass spectrometer

IGP	indole-3-glycerol phosphate
Ind	individual
JA	jasmonic acid
JAs	jasmonates
JMT	jasmonic acid carboxyl methyltransferase
LOX	13-lipoxygenase
$M^{+\bullet}$	molecular ion
MAP	mitogen activated protein
MAPK	mitogen-activated protein kinase
1-MCP	1-methylcyclopropene
MeJA	methyl jasmonate
MeSA	methyl salicylate
MKP	mitogen-activated protein kinase phosphatase
MS	mass spectrometer
MSTFA	*N*-methyl-*N*-trimethylsilyl-trifluoroacetamide
NMR	nuclear magnetic resonance
OPC 8:0	3-oxo-2-(2'(*Z*)-pentenyl)-cyclo pentane-1-octanoic acid
OPDA	12,13(*S*)-epoxy-octadecatrienoic acid
OPR	12-oxo-phytodieonic acid reductase
ORCA	octadecanoid-derivative responsive *Catharanthus* AP2-domain
P450	P450 monooxygenase
PI	proteinase inhibitor
PLA	phospholipase A
PLD	phospholipase D
r.t.	room temperature
SA	salicylic acid
SAM	*S*-adenosylmethionine
SAR	systemic acquired resistance
SIPK	salicylic acid-induced protein kinase
SMT	salicylic acid carboxyl methyltransferase
Tab	table
TMTT	(3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (C_{16}-homoterpene)
t_{ret}	retention time
TPS	terpene synthase
VOC	volatile organic compound
WIPK	wounding-induced protein kinase

Symbols and letters used in statistics

GLM	general linear model
LSD	Fisher's protected least significant differences test (i.e. post-hoc test)
n	sample size
P	probability
r	Pearson product moment correlation (i.e. measure of correlation; varies from -1 to 0)
SEM	standard error of the mean
SD	standard deviation
*	indicates a significant result ($P < 0.05$)
**	indicates a significant result ($P < 0.01$)
***	indicates a significant result ($P < 0.001$)

Abbreviations used for NMR assignment

d	dublett
q	quartett
δ	chemical resonance shift
s	singulett
t	triplett

1. General introduction

Ever since the greening of a strip of equatorial coastline that fringed tropical oceans approximately 425 million years ago, both plants and animals have expanded their ecological reach on land. Today their terrestrial representatives constitute the overwhelming bulk of macroscopic diversity on the planet which consists of more than 30 million species with many of them being as yet unstudied and unnamed. Two groups, namely flowering plants (Angiosperms) and insects (Insecta), contribute preponderance to the terrestrial macroscopic diversity we can observe to date.

If the earliest forms of land plants and insects are included, plants and insects have coexisted for as long as 350 million years and have, since then, developed myriads of relationships. The extraordinary radiation of flowering plants for example was clearly the impact of a coevolutionary interaction with pollinating insects (REGAL, 1977). In the Cretaceous, about 100 million years ago, when the first widely foraging seed dispersers came into existence (initially birds), insect pollination allowed flowering plants to produce outcrossed offspring with conspecifics, even when they were patchy and widely dispersed. Plants offered nectar to insects, thus creating new niches for nectivores with this diversification in turn creating new evolutionary possibilities for the plants. A permanent reciprocal selection pressure between pairs of single or multiple species led to coadaptation in which the evolving parties continually responded and counter-responded to the selection pressures imposed by each other. Such positive feedback loops caused rapid evolutionary changes in the interacting partners and thus enhanced speciation (LUNAU, 2004). Besides relationships which are mutually beneficial, such as between plants and their insect pollinators, the most common interaction involves insects preying on plants, and plant defences against herbivorous insects. This predator-host relationship is so common that virtually every plant species is preyed on by at least one insect species, and, according to the co-evolutionary theory of EHRLICH and RAVEN (1964), insect feeding on plants has been a determining factor in increasing species diversity in both herbivores and hosts (HARBORNE, 1988). On the basis of this long-standing relationship, it is not surprising that the strategies employed by plants that attempt to resist or evade their insect herbivores are very diverse.

Plant defence reactions

The spectrum of defensive mechanisms that plants have evolved against herbivores has been classified as direct (bottom-up) or indirect defences (top-down). Direct defences prevent herbivores from feeding via physical barriers, such as spines, thorns, trichomes, and waxes or chemically, via the production of toxins or anti-digestive and anti-nutritive com-

pounds. Additionally, plants may express traits that facilitate top-down control over the herbivore population by attracting natural enemies of the herbivore that indirectly defend them against their attackers (i.e. indirect defences, see Box 1). Direct and indirect defences can be expressed constitutively (i.e. permanently) or be induced upon mechanical wounding or herbivore damage.

Defence responses channel resources from vegetative and reproductive growth into protective mechanisms. Therefore, the plant must attain a balance to ensure survival from immediate and subsequent attacks without sacrificing plant vitality and reproduction. The finding that many direct and indirect defences are inducible suggests that these defences may incur costs to the plant. Fitness costs can arise directly from 'internal' mechanisms such as allocation costs, genetic costs and autotoxicity costs (i.e. the resistance trait itself is toxic to the plant) or indirectly from ecological interactions with mutualists or antagonistic species (HEIL & BALDWIN, 2002; STRAUSS *et al.*, 2002). These costs have been discussed as a driving force for the evolution of induced resistance (HEIL, 2001; HEIL & BALDWIN, 2002).

In order to minimize costs associated with induced resistance, plants must be able to identify their attacker to mount the most efficacious defence strategy. Indeed it has been shown that plants may tailor their defence response to the particular herbivore as has been shown on the level of transcriptional responses (VOELCKEL & BALDWIN, 2004), signal molecules (e.g. jasmonic acid: HALITSCHKE *et al.*, 2001; SCHITTKO *et al.*, 2000; ethylene: KAHL *et al.*, 2000) and defence-related metabolites such as e.g. nicotine (WINZ & BALDWIN, 2001), proteinase inhibitors (STOUT *et al.*, 1994; TAMAYO *et al.*, 2000), as well as volatile organic compounds (VOCs) (DE MORAES *et al.*, 1998; TAKABAYASHI *et al.*, 1995). Elicitation and orchestration of such complex biochemical and physiological responses requires at first the recognition of the currently feeding herbivore via physical and chemical signals which activate subsequent signal transduction cascades and finally up-regulate defence-related genes.

Herbivore recognition and signal transduction

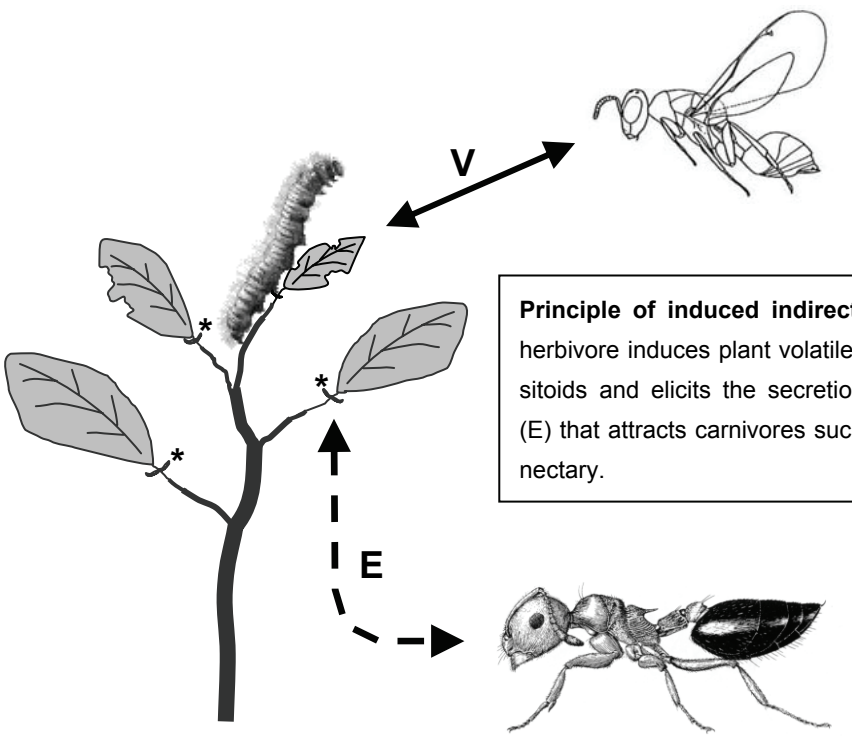
The study of how plants recognize an attacking insect herbivore has mainly focused on the emission of induced volatiles. Here it could be shown that the signals generated by the herbivore are first the mechanical damage that is inflicted, and second the chemical compounds of the herbivore's oral secretions which are introduced at the site of wounding.

Mechanical damage triggers the release of several VOCs. Immediately after wounding, the so-called green-leaf odours are released (i.e. saturated and un-

saturated C_6-carbon aldehydes, esters, and alcohols), which are produced by the autolytic oxidative breakdown of membrane lipids (PARÉ & TUMLINSON, 1999).

Box 1: Indirect defences

Indirect plant defences are traits that attract carnivorous arthropods to the trait-expressing plant to increase the predation pressure on herbivores (KESSLER & BALDWIN, 2001) and thus enhance plant fitness (FRITSCHE-HOBALLAH & TURLINGS, 2001; VAN LOON *et al.*, 2000). Attraction of beneficial arthropods to the plant may be actively mediated by rewards such as attractive food sources like *extrafloral nectar* (EFN; KOPTUR, 1992) and *food bodies* (JANZEN, 1966; RICKSON, 1971) or the provision of shelter in so-called *leaf domatia* (AGRAWAL & KARBAN, 1997). Furthermore, herbivore feeding can induce *de novo* production and emission of a highly specific blend of volatile organic compounds (VOCs) that may be used by carnivorous arthropods as a host-location cue (KESSLER & BALDWIN, 2001; TURLINGS *et al.*, 1990). In this case, the herbivore itself serves as a reward for the attracted plant defender. Indirect defences can be expressed *constitutively* (i.e. permanently) or be *induced* after herbivore damage. Induced indirect defences are mainly the secretion of EFN and the emission of VOCs. Other indirect defences, such as the formation of food bodies or leaf domatia, are often involved in obligate ant-plant interactions and thus expressed constitutively (i.e. myrmecophytism; for review see HEIL & MCKEY, 2003).



Principle of induced indirect plant defences: An herbivore induces plant volatiles (V) that attract parasitoids and elicits the secretion of extrafloral nectar (E) that attracts carnivores such as ants. * Extrafloral nectary.

Sources: Ant (*Crematogaster laeviscula* var. *clara* MAYR) from SMITH (1947), caterpillar (*Macrothyliacia rubi* LINNAEUS) from HONOMICHL *et al.* (1996), and wasp (*Austrotoxeuma kuscheli* BOUCEK) from NOYES (2003).

Some plant species (e.g. cotton, *Gossypium hirsutum*) also maintain chemical reserves such as trichomes or specialized glands, in which compounds accumulate to high levels and are released upon cell-wall disrupture (GANG *et al.*, 2001; PARÉ & TUMLINSON, 1997). Usually the bouquets emitted after mechanical damage are much weaker than what is emitted upon herbivore damage (MATTIACCI *et al.*, 1994; PARÉ & TUMLINSON, 1997; TURLINGS *et al.*, 1990). One explanation for this observation could be that plants are able to discriminate between a single event of mechanical damage and continuous feeding of a folivore. Indeed, adjusting the process of mechanical wounding to mimic the feeding mode of natural herbivores in terms of damage time and damaged leaf-area provoked a VOC emission which strongly resembled the bouquet induced by herbivores (MITHÖFER *et al.*, 2005).

Chemical elicitors (i.e. compounds that trigger a certain plant response) of VOC emission which have been isolated from the oral secretion or regurgitate of phytophagous insects comprise certain enzymes (FELTON & EICHENSEER, 1999; FUNK, 2001; MATTIACCI *et al.*, 1995) or fatty acid-amino acid conjugates (FACs) such as e.g. volicitin (ALBORN *et al.*, 1997). Volicitin which was originally isolated from corn (*Zea mays*) shows a VOC-inducing activity when added to mechanically wounded corn plants (ALBORN *et al.*, 1997). However, Lima bean leaves do not respond to volicitin or other FACs (SPITELLER *et al.*, 2001), indicating the

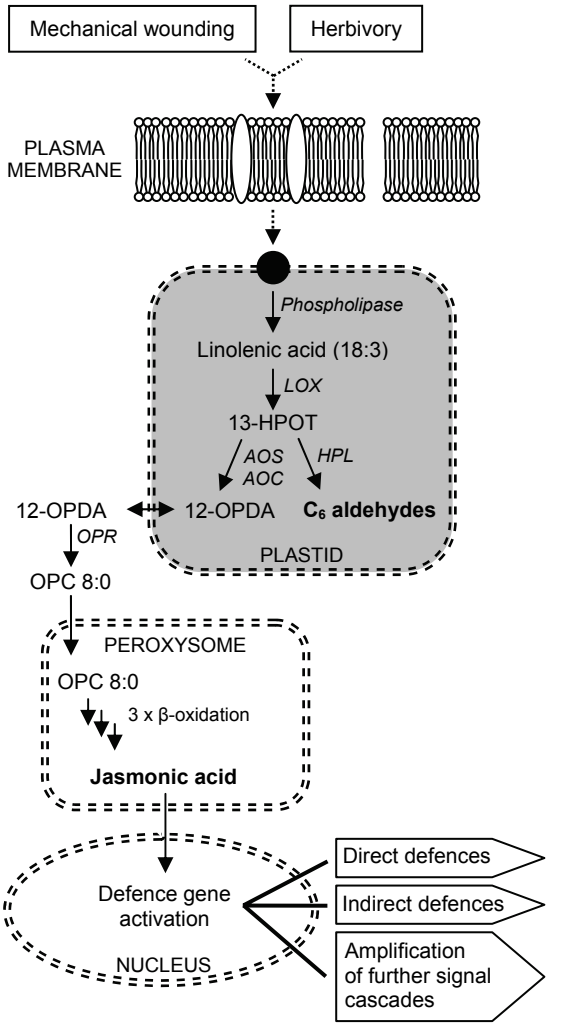


Figure 1. Schematic overview over the biosynthesis of jasmonic acid (octadecanoid pathway) and green leaf volatiles (C_6 aldehydes). Abbreviated *enzymes* involved are *LOX, 13-lipoxygenase; AOS, allene oxide synthase; AOC, allene oxide cyclase; OPR, 12-oxo-phytodieonic acid reductase; HPL, 13(S)-hydroperoxy-hydroperoxid lyase.* Substrates are 13-HPOT, 13(*S*)-hydroperoxy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid; 12-OPDA, 9(*S*)/13(*S*)-12-oxo-phytodienoic acid; OPC 8:0, 3-oxo-2-(2'-pentenyl)-cyclopentane-1-octanoic acid.

involvement of specific recognition mechanisms (TRUITT *et al.*, 2004).

Both physical and chemical signals are perceived at the outer membranes of the damaged cell layers, thus generating secondary signals such as a depolarization of the membrane potential (MAFFEI *et al.*, 2004), an intracellular calcium influx (ZIMMERMANN *et al.*, 1999), the generation of reactive oxygen species (OROZ-CO-CÁRDENAS *et al.*, 2001) or the activation of protein kinase/phosphatase cascades (KODAMA *et al.*, 2000; SEO *et al.*, 1995). Further cell signalling cascades allow the amplification of these signals as well as a regulation of the defence response in local (i.e. site of attack) and systemic tissues (i.e. remote, undamaged tissues). The signalling molecules involved in the downstream responses are mainly peptides (e.g. systemin) and phytohormones like jasmonic acid (JA), salicylic acid (SA), ethylene (ET) as well as lipid-derived oxylipins. Among them, JA and its derivatives, collectively called jasmonates (JAs), are of central importance for the regulation of both direct and indirect plant defence responses against wounding and herbivory (HALITSCHKE & BALDWIN, 2004). This has been elegantly demonstrated by BALDWIN and co-workers who silenced the lipoxygenase3 gene in the wild tobacco (*Nicotiana attenuata*), rendering the transformed plants impaired in oxylipin signalling and thus unable to activate their direct and indirect anti-herbivore defences. When planted into native habitats, lipoxygenase-deficient plants were more vulnerable to adapted herbivores and attracted novel herbivore species, underlining the key role of the octadecanoid pathway (Fig. 1) for plant defence and host selection of herbivores (KESSLER *et al.*, 2004).

Interactions of the octadecanoid pathway with additional signalling cascades, which can be either synergistic or antagonistic, help the plant to prioritise the activation of a particular defence pathway over another one. For instance SA, which is known to be involved in mediating the development of systemic acquired resistance (SAR) in response to pathogen attack, acts antagonistically to JA (STOUT *et al.*, 1999; THALER *et al.*, 1999). Signalling compounds may also differentially affect direct and indirect defences: ET for example can suppress the induction of direct defence without negative effects on the induction of indirect defence (KAHL *et al.*, 2000). This complex and fine-tuned interplay of different signal transduction pathways which is still not perfectly understood, represents an adaptive tailoring of a plant's defence response against a particular attacker.

Ecology of indirect defences

Even if mechanistic knowledge on endogenous processes associated with herbivore attack is accumulating, the ecology of indirect defences is still relatively poor

understood. Herbivore-induced plant volatiles, for example, may affect many organisms in the environment of the emitting plant such as herbivores, carnivores or pollinators. So far, these interactions have been mainly studied under laboratory conditions, thereby focussing on the behavioural and electrophysiological responses of herbivores and carnivores to the VOCs emitted from various herbivore-infested plant species. These analyses revealed that the released VOC bouquets can be used by arthropod predators and parasitoids to locate their particular hosts or prey (for review see TURLINGS & BENREY, 1998; VAN POECKE & DICKE, 2004). In some cases, the released VOCs also functioned as a direct defence by repelling herbivores (BERNASCONI *et al.*, 1998; DICKE, 1986; LANDOLT, 1993) or reducing herbivore performance (TURLINGS & TUMLINSON, 1991). However, the few arthropod species which were involved as herbivores and carnivores in these investigations represent a restricted range of feeding guilds. Moreover, in most of the cases, the arthropod species used did not co-occur with the focal plant species in nature, or it remained unclear whether the investigated plant-animal interaction was also of significance in the wild. Among the field studies, which are available to date on the attraction of carnivores to VOCs emitted from herbivore-infested plants, only one has been performed on naturally growing plant populations (KESSLER & BALDWIN, 2001) and not in simplified agro-ecosystems (DE MORAES *et al.*, 1998; DRUKKER *et al.*, 1995; SHIMODA *et al.*, 1997). This lack emphasizes the need for field studies on the ecological relevance of herbivore-induced volatile organic compounds (HI-VOCs) for the plant as well as for the arthropods involved in the interaction as both herbivores and carnivores.

The situation is different for extrafloral nectar (EFN). The first ecological field experiments on this indirect defence trait had been conducted already in 1889: VON WETTSTEIN excluded ants from *Jurinea mollis*, thereby demonstrating that nectary-visiting ants reduce levels of plant damage. Since then, the ant-plant protection hypothesis has been repeatedly tested. The majority of these studies has shown that ants benefit plants, yet with the magnitude of the benefit varying greatly, depending on factors such as the need for mutualistic ants or a varying abundance and identity of ant species (for review see BRONSTEIN, 1998; HEIL & MCKEY, 2003; OLIVEIRA & FREITAS, 2004). However, not only ants, but also other arthropods such as wasps (BUGG *et al.*, 1989; STAPEL *et al.*, 1997), flies (HEIL *et al.*, 2004a), or spiders (RUHREN & HANDEL, 1999) are attracted to the secreted EFN. Whether these non-ant visitors of extrafloral (EF) nectaries also protect the plant or act as parasites, because they consume EFN without contributing to plant

fitness, remains largely unclear. Among the first reports that considered multi-species interactions between plants and EF nectary-visiting arthropods is an elegant field study of CUAUTLE and RICO-GRAY (2003), which demonstrated that both ants and wasps positively affect plant fitness. This study highlights the need to take not only ants, but also other predators and parasitoids into account when the effectiveness of EFN as an indirect defence (PEMBERTON & LEE, 1996) is estimated.

Inter-plant signalling

HI-VOCs provide chemical information on the status of attack of the emitting plant which might be used not only by higher trophic levels, but also by neighbouring plants of the same or other species. Two decades ago, researchers indeed reported that wounding or herbivore attack resulted in an altered herbivore resistance with detrimental effects on populations of insect herbivores not only of the attacked plants, but also of plants growing nearby (BALDWIN & SCHULTZ, 1983; RHOADES, 1983). In some of these experiments, an aerial transfer of information was the most parsimonious explanation for the observed phenomena. Since then, the idea of 'talking trees' that convey the information about their state of attack to their undamaged neighbour that in turn 'eavesdrops' on these cues and responds with the induction of its own defence (Fig. 2), has captured the imagination of many researchers.

Initially harshly criticised for limited replication and the failure to consider alternative hypotheses (FOWLER & LAWTON, 1985), major advances in recent years have considerably supported the hypothesis that plants can adaptively respond to chemical information emitted from their neighbour. Experimental evidence suggests that eavesdropping between plants may occur: Exposing plants to increased levels of putative volatile signals indicated transcriptional changes of defence-related genes (ARIMURA *et al.*, 2005; ARIMURA *et al.*, 2001; ARIMURA *et al.*, 2000b; BATE & ROTHSTEIN, 1998; PENG *et al.*, 2005) as well as changes in the abundance of phytohormones (ARIMURA *et al.*, 2002; ENGELBERTH *et al.*, 2004) and defence-related metabolites such as terpenoids (ENGELBERTH *et al.*, 2004), proteinase inhibitors (FARMER & RYAN, 1990; TSCHARNTKE *et al.*, 2001) or phenolic compounds (BALDWIN & SCHULTZ, 1983). However, most of these studies were conducted under artificial laboratory conditions, while rigorous verification of the plant-plant communication-hypothesis under field conditions was largely lacking (BRUIN & DICKE, 2001). Among the studies addressing this issue, DOLCH and TSCHARNTKE (2000) could show that partial defoliation of alder trees (*Alnus glutinosa*) to simulate herbivory resulted in an induced resistance in the

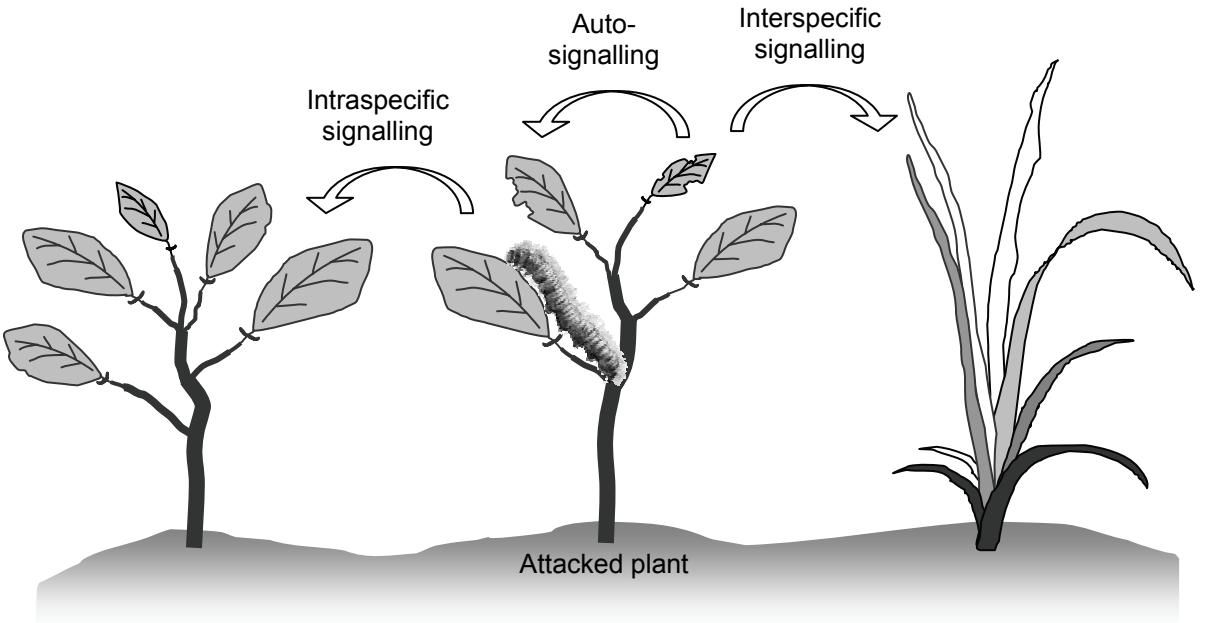


Figure 2. Three modes of airborne signalling from or within herbivore-damaged plants are indicated: signalling to undamaged congeners, signalling to members of other species, and auto-signalling outside the plant body. Arrows represent herbivore-induced plant volatiles. Modified after FARMER (2001). Source: caterpillar (*Macrothylia rubi*) from HONOMICHL *et al.* (1996).

defoliated trees as well as in conspecific neighbours. More detailed analyses in the laboratory identified changes in the leaf chemistry as possible responses causal for the observed resistance (TSCHARNTKE *et al.*, 2001). In another field study, experimental clipping of sagebrush increased the herbivore resistance of neighbouring tobacco plants, which resulted in a reduced herbivory and an increased life-time seed production compared to control plants next to undamaged sagebrush plants (KARBAN *et al.*, 2000; KARBAN & MARON, 2002). Here, the biologically active enantiomer (3*R*,7*S*) methyl jasmonate has been proposed as the aerially transmitted signal that caused an elevated activity of an anti-nutritive enzyme (polyphenol oxidase) in downwind tobacco plants (KARBAN *et al.*, 2000).

So far, the study of KARBAN and colleagues (2000) is the only to show the VOC-mediated expression of defence-related plant metabolites in undamaged downwind plants under field conditions. Furthermore, most studies on plant-plant communication focused on the induction of direct defences, ignoring the possibility that also indirect plant defences may be affected.

*The Lima bean (*Phaseolus lunatus*) - A model system in chemical ecology*

Like many other Fabaceae, Lima bean has evolved cyanogenic glycosides as a direct defence against herbivores (BALLHORN

et al., 2005). Tissue break-down exposes the vacuole-located glucosides to β-glucosidases and hydroxy-nitrile lyases, with subsequent hydroylsis leading to the release of cyanide, which is a universal respriratory poison (BENNETT & WALLS-GROVE, 1994).

The reason, however, why Lima bean became an attractive model system for chemical ecologists, is the observation that it emits significantly increased amounts of about 10 different VOCs after herbivore damage. These are likely to be synthesized *de novo* (BOLAND *et al.*, 1995; BOUWMEESTER *et al.*, 1999; DONATH & BOLAND, 1995) and are derived from the lipoxygenase pathway, the shikimic acid pathway, and the isoprenoid pathway (DICKE *et al.*, 1999; for details on the pathways involved see manuscript I, Fig. 3). Like in many other plant species, herbivore damage in Lima bean is accompanied by a transient increase in endogenous JA-levels (KOCH, 2001). Exogenous application of JA (DICKE *et al.*, 1999; HOPKE *et al.*, 1994; OZAWA *et al.*, 2000a) or the early intermediates of jasmonate biosynthesis α-linolenic acid and OPDA (KOCH *et al.*, 1999) to plants also results in the emission of VOC blends which are similar, yet not identical to those released after herbivore damage. VOCs released from herbivore-infested or JA-treated Lima bean plants have been shown to exhibit an attractive effect not only on predatory arthropods such as mites (SABELIS & VAN DE BAAN, 1983), thrips (SHIMODA *et al.*, 1997), staphylinid beetles (SHIMODA *et al.*, 2002) and braconid wasps (PETITT *et al.*, 1992), but also on herbivorous beetles (HEIL, 2004b). Moreover, VOCs may deter herbivores such as chrysomelid beetles (HEIL, 2004b) and thus act as a direct defence.

In addition to the herbivore-induced emission of VOCs, Lima bean bears extrafloral (EF) nectaries (Fig. 3). These specialized, nectar-secreting organs are located on its bracts, or arranged pairwise at the stipules of the trifoliate leaves as well as at the petioles of the individual

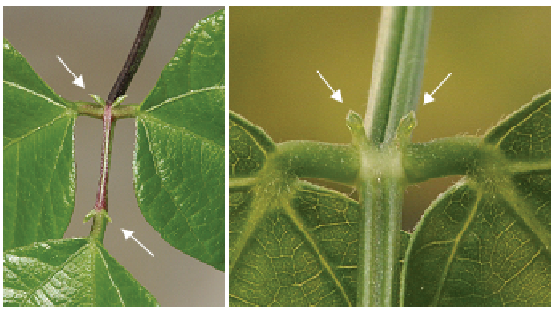


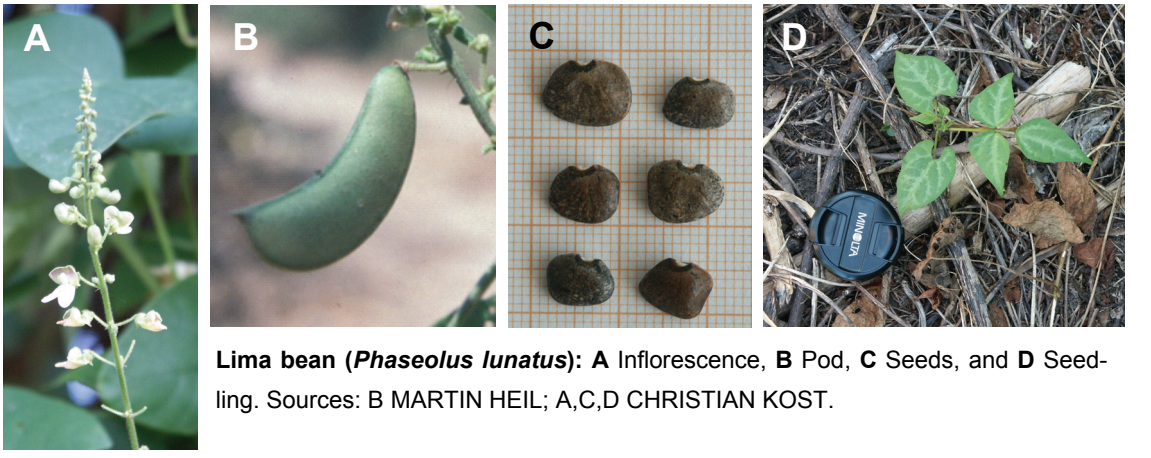
Figure 3. Location of the extrafloral nectar-secreting stipules (i.e. extrafloral nectaries) within a trifoliate leaf of *Phaseolus lunatus*. Source: CHRISTIAN KOST.

leaflets (HEIL, 2004a). The secretion rate of EFN is also inducible by JA, thus providing the Lima bean with the two inducible, indirect defences: emission of HI-VOCs and secretion of EFN. Besides few other plant species such as e.g. broad bean (*Vicia faba*; COLAZZA *et al.*, 2004; FIGIER, 1971) or cotton (PARÉ & TUM-

LINSON, 1997; WÄCKERS & BONIFAY, 2004) for which both indirect defences have been described, this combination of traits makes Lima bean an ideal system

Box 2: The natural history of Lima bean

Lima bean (*Phaseolus lunatus* LINNAEUS, Fabaceae) is a food legume that originated in Central and South America and is now cultivated in all tropical regions. Studies based on morphological and biochemical observations (DEBOUCK *et al.*, 1989; GUTIÉRREZ SALGADO *et al.*, 1995; MAQUET *et al.*, 1990) as well as on molecular data (FOFANA *et al.*, 2001; FOFANA *et al.*, 1997) indicate the existence of two distinct gene pools: the 'Mesoamerican' type being widely distributed in neotropical lowlands, while the 'Andean' type appears to be restricted to the western Andes. Since both gene pools comprise closely related wild and cultivated forms, two independent domestication events were suggested (GUTIÉRREZ SALGADO *et al.*, 1995). According to AFLP analyses (i.e. *amplified fragment length polymorphism*, a method to examine variation in DNA sequences), the variety 'Jackson Wonder Bush' that is used in many laboratory studies as well as the wild type plants that have been used in all of my experiments belong to the Mesoamerican genotype (M. HEIL, unpublished data). The wild forms are self-compatible with a mixed mating system, i.e. predominantly self-pollinating but with a fair amount of outcrossing (HARDING & TUCKER, 1969; ZORRO BI *et al.*, 2005). Plants in natural populations grow as annuals or short-living perennials, although the aboveground parts usually die during the dry season. Wild Lima bean forms soil seed banks with the dormancy of seed being broken by drastic temperature changes and high precipitation rates (DEGREEF *et al.*, 2002): In June or July the plants start to germinate or bud and the first inflorescences usually appear in October or November. Depending on water supply, the production of flowers and fruits (see below) ends between February and April (HEIL, 2004a).



Lima bean (*Phaseolus lunatus*): A Inflorescence, **B** Pod, **C** Seeds, and **D** Seedling. Sources: B MARTIN HEIL; A,C,D CHRISTIAN KOST.

Figure 4. Lima bean (*Phaseolus lunatus*) growing in its natural habitat. Wild plants entwine trees and shrubs along a roadside near Puerto Escondido, Mexico. Source: CHRISTIAN KOST.

to study the importance of these two defensive traits in nature (see Box 2 for information on the natural history of the Lima bean).

Aim of this thesis

Most of the knowledge on the ecology of HI-VOCs released from Lima bean has been derived from laboratory-based approaches using Lima bean seedlings. A look at the size and growth structure of Lima bean growing in nature (Fig. 4) leaves uncertainty as to which extend the results gathered under artificial laboratory conditions are also valid for the natural situation in the field. Moreover, the existence of EF nectaries in Lima bean has just recently been discovered (HEIL, 2004a), thus nearly no ecological information is available on this trait until now. These shortcomings highlight the need for evaluating the role of both indirect defensive traits under natural conditions. This should be realized in the present thesis.

In a pioneering study, HEIL (2004a) could experimentally demonstrate that wild growing Lima bean plants, which have been repeatedly induced with exogenous application of JA, responded to this treatment with an increased seed set. Since the JA-treatment induced both the emission of VOCs and the secretion of EFN, it remained unclear how the two indirect defences had contributed to the observed beneficial effect. Therefore, this

study provided an ideal starting point for further field experiments.

Based on the findings of HEIL (2004a), the present work applies an integrative approach, in which the function of the two indirect defences, emission of VOCs and secretion of EFN, is analysed on the level of i) the trait-expressing plant, ii) the attracted arthropod community, and iii) conspecific plant neighbours. In detail, the following three main sets of problems were addressed from a phytocentric perspective:

(1) How effective are the two inducible, indirect defences - VOC emission and EFN secretion - at the natural growing site of Lima bean in Mexico? Do wild plants of *P. lunatus* benefit from either VOCs or EFN alone under natural growing conditions?

(2) Which animals are involved in the tritrophic interaction as plant defenders? Which animals are attracted to VOCs and which to EFN?

(3) Is there a VOC-mediated interaction between herbivore-damaged and undamaged Lima bean plants? Does the emission of HI-VOCs induce the secretion of EFN in undamaged, neighbouring tendrils? Which compounds within the complex blend of HI-VOCs are responsible for the induction of EFN secretion?

2. Thesis outline – List of manuscripts and author's contribution

Manuscript I

Herbivore-induced, indirect plant defences

Gen-ichiro Arimura, Christian Kost, and Wilhelm Boland

Biochimica et Biophysica Acta (2005) **1734,** 91-111

This review summarizes the current knowledge on herbivore-induced, indirect plant defences on the levels of signal perception and transduction, gene expression and biosynthesis of defence-related metabolites as well as it examines indirect defences in the light of their ecology and evolution. Focussing on the two defensive traits emission of herbivore-induced plant volatiles (HIPVs) and secretion of EFN, it provides a comprehensive overview over the latest developments in the field and highlights perspectives for future research. Thus this review provides the theoretical background of the thesis.

The article was a joint effort by all three co-authors. I was responsible for writing the 'Abstract', the 'Introduction' (section 1), the sections 'Ecology of induced indirect defences' (section 4) and 'Conclusions' (section 5) as well as for the preparation of table 1 and Fig. 5. The final draft of the article was optimized in agreement with all three co-authors.

Manuscript II

Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (*Phaseolus lunatus*, Fabaceae)

Christian Kost and Martin Heil

Basic and Applied Ecology (2005) **6**, 237-248

This manuscript describes field experiments on the defensive effect of EFN in *Phaseolus lunatus*, identifies putative plant defenders and examines insect responses towards an artificial mixture of EFN. The results of this study suggest that Lima bean benefits of increased amounts of EFN and that ants and also flying defenders like wasps or flies contribute to the observed beneficial effect.

I was responsible for experimental work, data evaluation, arthropod identification, and statistical analysis. MARTIN HEIL helped with the establishment of the experimental plots, counting of plant defenders, and estimation of the herbivory rate. Analysis of EFN composition was done by JANINE RATTKE and me and MANFRED VERHAAGH determined the collected ant species. I wrote the first draft of the manuscript, which was refined in collaboration with MARTIN HEIL.

Manuscript III

Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants

Christian Kost and Martin Heil

Journal of Ecology, accepted

This manuscript analyses whether plant-plant communication can occur in Lima bean under natural growing conditions and whether this has defensive effects for the receiver of the signal. In several independent experiments an inductive effect of entire volatile blends and of single blend constituents on the EFN secretion rate of conspecific plants is verified. Furthermore, the long-term consequences of a volatile-induced EFN secretion are assessed on the level of fitness-relevant plant parameters and number of plant defenders attracted, and compared to the defensive effect of EFN alone (manuscript II).

I had the idea of a potential induction of EFN by HI-VOCs. The planning and realization of the nectar-induction experiments as well as the chemical and statistical analysis were done by me. The experimental design of the long-term experiment was a joint effort by MARTIN HEIL and me. MARTIN HEIL also helped with the establishment of the experimental plots, counting of plant defenders, and estimation of fitness-relevant plant parameters. Organic synthesis of VOCs was done by me. SVEN ADOLPH and MESMIN MEKEM SONWA helped with the development and optimization of synthesis strategies. The manuscript was written by me, optimized after suggestions of MARTIN HEIL.

Manuscript IV

The defensive value of two indirect defences of Lima bean in nature

Christian Kost and Martin Heil

In preparation for *Oecologia*

This manuscript, which is based on field experiments at the Lima bean's natural growing site, contrasts the defensive effect of EFN secretion and VOC emission. In groups of neighbouring tendrils the amount of EFN and VOCs were artificially increased and compared to JA-treated and control tendrils. The development of fitness-relevant plant parameters was monitored as well as the arthropod community visiting the studied plants was assessed by counting and sticky-trap captures. The interaction of the two indirect defences is discussed in the light of the functional groups of arthropods attracted.

The experimental design was a joint effort by me and MARTIN HEIL who also helped with the establishment of the experimental plots, counting of plant defenders, and estimation of fitness-relevant plant parameters. I was respon-sible for preceding experiments on the emission of VOCs out of lanolin paste, the realisation of the field experiments as well as the statistical data analysis. Organic synthesis of VOCs was done by me with support of SVEN ADOLPH and MESMIN MEKEM SONWA. The manuscript was written by me and optimized after suggestions of MARTIN HEIL.

Manuscript V

Dehydrogenation of ocimene by active carbon: Artefact formation during headspace sampling from leaves of *Phaseolus lunatus*

Mesmin Mekem Sonwa, Christian Kost, Anja Biedermann, Robert Wegener, Stefan Schulz, and Wilhelm Boland

Submitted to *Tetrahedron*

This manuscript examines the observation that during volatile-collection of induced Lima bean plants, two compounds can only be detected when the headspace is sampled with charcoal traps, but not when other adsorption materials such as e.g. SPME or Tenax are used. It is shown that ocimene, a major constituent of the plant's volatile blend, reacts with the active carbon of the trapping adsorbant leading to artefact formation. A mechanism for this chemical reaction is proposed as well as an exploitation of this new reaction for a rapid functionalisation of ocimene is verified.

I discovered the phenomenon and performed the experiment in which different VOC-sampling methods are compared (Tab. 1). The experiments on the mechanistic elucidation (radical versus hydride) were performed by MESMIN MEKEM SONWA. Experiments on the functionalisation of ocimene with different solvents and with DDQ (3.7.5) were done by ANJA BIEDERMANN and me. Writing of the manuscript was a joint effort of MESMIN MEKEM SONWA, WILHELM BOLAND and me.

Manuscript I

Herbivore-induced, indirect plant defences

Gen-ichiro Arimura, Christian Kost, Wilhelm Boland*

Biochimica et Biophysica Acta (2005) **1734**, 91-111

Accepted: 1 March 2005

Department of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Beutenberg Campus
Hans-Knöll-Str. 8
D-07745 Jena, Germany

*** Corresponding author:**
Wilhelm Boland
Department of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Beutenberg Campus, Hans-Knöll-Str. 8
D-07745 Jena, Germany
Phone: + + 49 - 3641 - 57 12 00
Fax: + + 49 - 3641 - 57 12 02
E-mail: boland@ice.mpg.de

Abstract

Indirect responses are defensive strategies by which plants attract natural enemies of their herbivores that act as plant defending agents. Such defences can be either constitutively expressed or induced by the combined action of mechanical damage and low- or high-molecular weight elicitors from the attacking herbivore. Here, we focus on two induced indirect defences, namely the de novo production of volatiles and the secretion of extrafloral nectar, which both mediate interactions with organisms from higher trophic levels (i.e. parasitoids or carnivores). We give an overview on elicitors, early signals, and signal transduction resulting in a complex regulation of indirect defences and discuss effects of cross-talks between the signalling pathways (synergistic and antagonistic effects). In the light of recent findings, we review molecular and genetic aspects of the biosynthesis of herbivore-induced plant volatiles comprising terpenoids, aromatic compounds, and metabolites of fatty acids which act as infochemicals for animals and some of which even induce defence genes in neighbouring plants. Finally, ecological aspects of these two indirect defences such as their variability, specificity, evolution as well as their ecological relevance in nature are discussed.

Key words: Extrafloral nectar; herbivore-induced plant volatile; indirect defence; oxylipin; signalling pathway

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ALA, alamethicin; CAM, calmodulin; CDPK, calcium-dependent protein kinase; DMNT, (*E*)-4,8-dimethyl-1,3,7-nonatriene; EAG, electroantennogram; EF, extrafloral; EFN, extrafloral nectar; ERF, ethylene-responsive transcription factor; FAC, fatty acid–amino acid conjugate; FAD, ω-3 fatty acid desaturase; FPP, farnesyl diphosphate; GPP, geranyl diphosphate; HIPV, herbivore-induced plant volatile; HPL, fatty acid hydroperoxide lyase; IGP, indole-3-glycerol phosphate; JA, jasmonic acid; JMT, JA carboxyl methyltransferase; LOX, lipoxygenase; MAPK, mitogen-activated protein kinase; 1-MCP, 1-methylcyclopropene; MeJA, methyl jasmonate; MKP, MAPK phosphatase; OPDA, 12-oxophytodienoic acid; ORCA, octadecanoid-derivative responsive *Catharanthus* AP2-domain; P450, P450 monooxygenases; PI, proteinase inhibitor; PLA, phospholipase A; PLD, phospholipase D; SA, salicylic acid; SAM, *S*-adenosylmethionine; SAR, systemic acquired resistance; SIPK, salicylic acid-induced protein kinase; SMT, SA carboxyl methyltransferase; TMTT, (3*E*,7*E*)-4,8,12-trimethyl-trideca-1,3,7,11-tetraene; TPS, terpene synthase; WIPK, wounding-induced protein kinase.

1. Introduction

Plants have evolved a wide spectrum of strategies to defend themselves against herbivores. Such defensive strategies can be classified as direct defences, which immediately exert a negative impact on herbivores, or indirect defences, which include higher trophic levels, thus fulfilling the defensive function (PRICE *et al.*, 1980). Direct defences may prevent herbivores from feeding via physical barriers, such as spines, thorns, trichomes, and waxes or chemical ones, with secondary plant metabolites (e.g. phenylpropanoids, terpenoids, alkaloids, and fatty acids); or via specialized defence proteins (e.g. proteinase inhibitors, so-called PIs). On the other hand, indirect defences work by attracting the herbivores' enemies, such as parasitoids or predators, which actively reduce the number of feeding herbivores. Both strategies can be either constitutive, meaning that they are always expressed, or inducible, meaning that they appear only when needed, namely, following herbivory.

In recent years especially, induced, indirect defences have received increasing attention and have been studied on the genetic, biochemical, physiological, and ecological levels. The aim of this review is to summarize the current state of knowledge, merge results from different biological fields, and point to new possibilities for future research. Beginning with the first contact between plant and herbivore, and including herbivore recognition, signal elicitation, and early signalling steps, and followed by a description of the signalling cascades and biosynthesis pathways involved, we will end with an overview of the ecology and evolution of induced, indirect defensive plant traits.

2. Cell- and long-distance signalling in plants in response to herbivory

Plants have evolved a large array of interconnected cell-signalling cascades, resulting in local resistance and long-distance signalling for systemic acquired resistance (SAR). Such responses were initiated with the recognition of physical and chemical signals of the feeding herbivores, activate subsequent signal transduction cascades, and finally lead to an activation of genes involved in defence responses that consequently enhance feedback signalling and metabolic pathways (Fig. 1). This section describes the aspects of cell signalling involved in the induction of indirect herbivore responses.

2.1. Elicitors

In the 1990s and earlier, many chemical ecologists believed that herbivorous oral secretions and regurgitants elicited insect-induced plant responses, since simple mechanical wound stimuli, in many cases, could not mimic plant responses following insect attack (BALDWIN, 1988; BOLAND *et al.*, 1992; PARÉ & TUMLINSON, 1997;

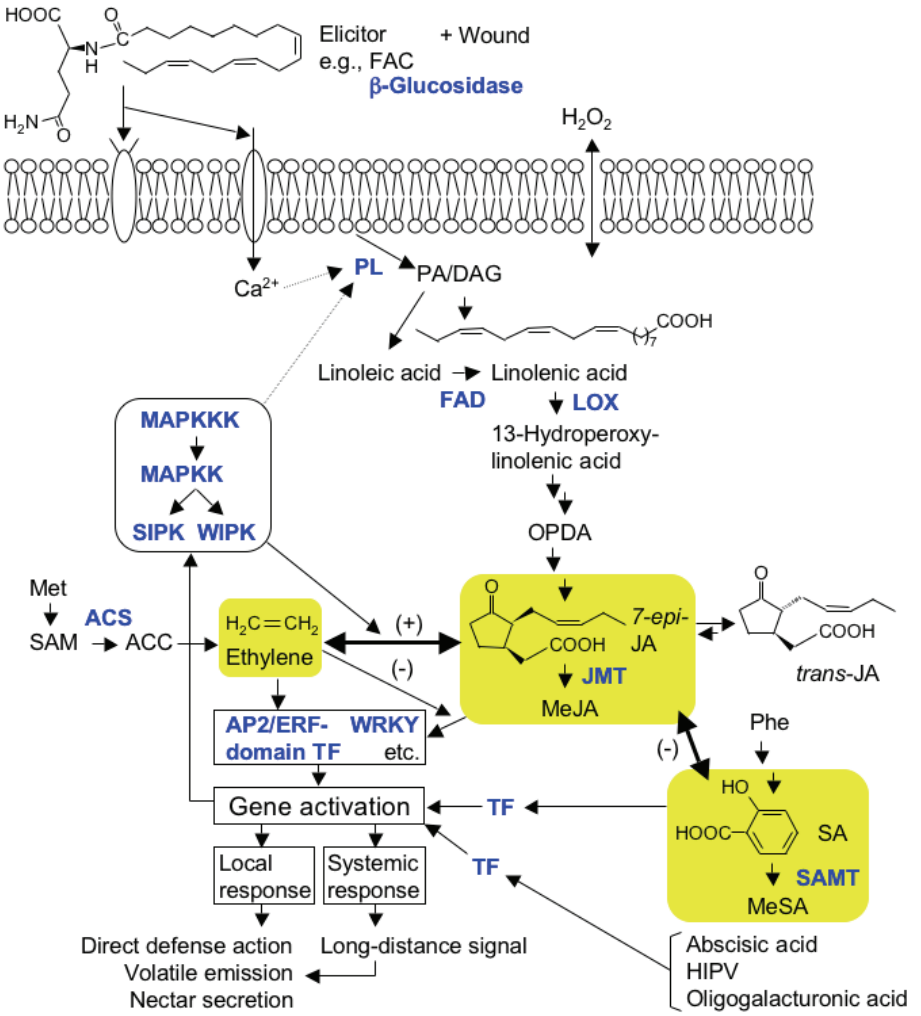


Figure 1. Schematic representation of the signalling pathways required for herbivore-induced responses in plants. This scheme merges the evidence obtained from several plant taxa. The overall scenario may differ in certain plants; in particular the existence and the extent of synergistic and antagonist interaction between pathways may vary significantly. Elements in blue represent enzymes. Broken arrows indicate possible steps not yet described. Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ACS, ACC synthase; DAG; diacylglycerol; FAC, fatty acid-amino acid conjugate; FAD, ω-3 fatty acid desaturase; HIPV, herbivore-induced plant volatiles; JA, jasmonic acid; JMT, JA carboxyl methyl transferase; LOX, lipoxygenase; MAPK, mitogen-activated protein kinase; MeJA, methyl JA; MeSA, methyl SA; OPDA, 12-oxophytodienoic acid; PL, phospholipase; PA, phosphatidic acid; SA, salicylic acid; SAM, *S*-adenosyl-methionine; SAMT, SA carboxyl methyl transferase; TF, transcription factor.

TURLINGS *et al.*, 1990). β-Glucosidase derived from regurgitate of *Pieris brassicae* larvae was identified as a potential elicitor of herbivore-induced plant volatiles (HIPVs) (MATTIACCI *et al.*, 1995). The application of this β-glucosidase to mechanically wounded parts of cabbage leaves resulted in the emission of a volatile blend that attracted parasitic wasps (*Cotesia glomerata*). On the other hand,

the application of glucose oxidase, a salivary gland enzyme, inhibited the synthesis of nicotine in tobacco leaves and may thus be responsible for the observed resistance of feeding herbivores (MUSSER *et al.*, 2002). The suppressive effect, which maintains the plant palatable, may be due to the products of glucose oxidase, namely hydrogen peroxide (H_2O_2) and gluconic acid.

N-(17-hydroxylinolenoyl)-L-glutamine (volicitin), a fatty acid-amino acid conjugate (FAC), was detected in the oral secretion of beet armyworm larvae (*Spodoptera exigua*) (ALBORN *et al.*, 1997) (Fig. 2). This FAC was identified as the main elicitor inducing the emission of a volatile blend in maize plants (*Zea mays*) similar to that emitted when caterpillars fed on them. While volicitin was isolated from *S. exigua*, other FACs with biological activity, such as *N*-acyl Gln/Glu, have been isolated from the regurgitate of several lepidopteran species (HALITSCHKE *et al.*, 2001; POHNERT *et al.*, 1999; SPITELLER & BOLAND, 2003). For example, the FAC *N*-linolenoyl-Glu in the regurgitate of the tobacco hornworm (*Manduca sexta*) was characterised as a potential elicitor of volatile emission in tobacco plants. FACs can induce the accumulation of 7-*epi*-jasmonic acid (JA) an octadecanoid-derived phytohormone that elicited transcripts of herbivore-responsive genes in the tobacco plants (HALITSCHKE *et al.*, 2001). 7-*Epi*-jasmonic acid is the originally

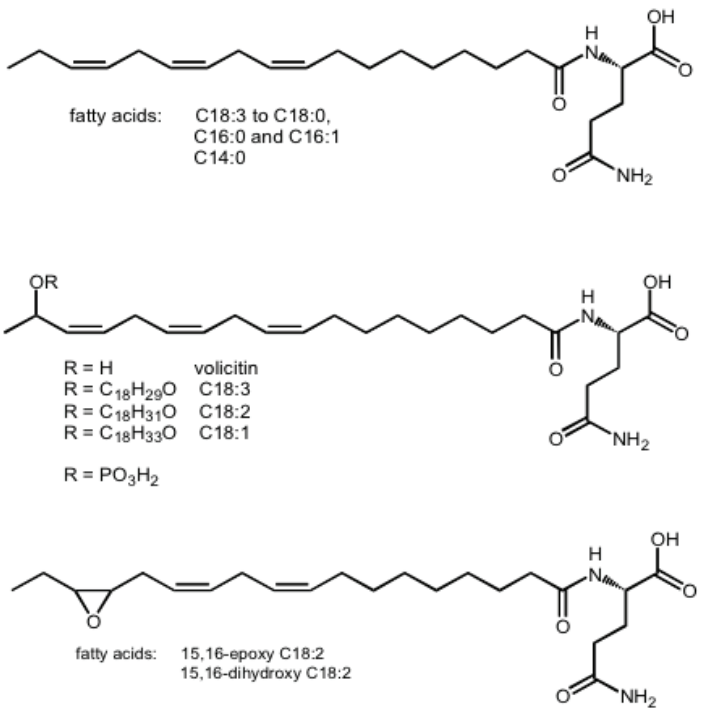


Figure. 2. *N*-acyl glutamines from oral secretions of lepidopteran larvae.

produced and biologically active form of JA, which is isomerised *in planta* to the less active *trans*-isomer (see Fig. 1). Recently, a plasma membrane protein from maize was suggested as a volicitin-binding protein (TRUITT *et al.*, 2004). Plants that have been either pre-treated with methyl jasmonate (MeJA) or *S. exigua* feeding showed increased binding activity of labelled volicitin to the plasma membrane. These findings suggest that volicitin-induced JA enhanced the interactions of the plasma membrane protein with volicitin as a ligand or the induced formation of the proteins after the recognition of volicitin. However, volicitin is not generally active. Leaves of Lima bean (*Phaseolus lunatus*), for example, do not respond to volicitin or to other FACs with the induction of volatile emission (SPITELLER *et al.*, 2001).

2.2. Early and secondary signal transduction pathways

After mechanical damage, reactive oxygen species were generated near cell walls of tomato vascular bundle cells and the resulting H_2O_2 was shown to act as a second messenger for the activation of defensive genes in mesophyll cells which are expressed later (OROZCO-CÁRDENAS *et al.*, 2001). In Lima bean leaves infested with *Spodoptera littoralis* larvae, a depolarization of the membrane potential (V_m) and intracellular calcium influx were observed only at the site of damage (MAFFEI *et al.*, 2004). Simple mechanical wounding of the leaves also caused V_m depolarization, yet without a concomitant influx of calcium. Hence, already at this early step of signal recognition and transduction, the pathways offer different responses to wounding and herbivore attack. Thus, both wounding and the introduction of herbivore-specific elicitors appear to be essential for the full induction of defence responses.

On the other hand, recent studies applying a continuous rather than a single instance of mechanical damage (pattern wheel) to Lima bean leaves clearly resulted in the emission of volatile blends resembling those that occur after herbivore damage (MITHÖFER *et al.*, 2005). As mentioned above, Lima bean and cotton (*Gossypium hirsutum*) do not respond to FACs with increased volatile emissions (SPITELLER *et al.*, 2001). This observation may be consistent with a high internal threshold for mechanical damage which, in some plant species, is reached only after continuous wounding and which triggers the typical repertoire of defence responses caused by elicitors. cDNA was isolated from a species of tobacco, encoding a mitogen-activated protein kinase (MAPK), whose transcript begins to accumulate in the leaves 1 min after a single mechanical wounding event (SEO *et al.*, 1995). This protein kinase (WIPK) is considered essential for JA formation and JA-induced responses in tobacco, since transgenic plants in which *WIPK* has been silenced

show impaired accumulation of JA and MeJA after wounding (SEO *et al.*, 1995). In contrast, the loss of *WIPK* function in this transgenic line resulted in an increased accumulation of salicylic acid (SA) and its sugar conjugate salicylic acid β-glucoside after wounding; however, such accumulation was not observed in wild-type plants. The signalling pathways associated with JA and SA are generally thought to cross-communicate antagonistically (see below). Moreover, WIPK has also been reported to elicit the transcription of a gene for a ω-3 fatty acid desaturase (FAD7), which catalyses the conversion of linoleic acid to linolenic acid, a precursor of JA (KODAMA *et al.*, 2000). Thus, WIPK may be an early activator of the octadecanoid pathway. WIPK and the SA-induced protein kinase (SIPK) were found to share an upstream MAPKK, NtMEK2 (LIU *et al.*, 2003), and to interact with the calmodulin (CaM)-binding MAPK phosphatase (NtMKP1) (YAMAKAWA *et al.*, 2004). Both kinases are commonly involved in wound-mediated defence responses in tobacco (LIU *et al.*, 2003; SEO *et al.*, 1999; ZHANG & KLESSIG, 1998). If the MAPK cascades in tobacco were only interlinked as described above, an active mutant of the upstream kinase of SIPK (*NtMEK2*^{DD}) should have increased the accumulation of JA and MeJA, but it did not (KIM *et al.*, 2003). Instead, ethylene was assumed to be involved in plant defence responses mediated by the NtMEK2-SIPK/WIPK pathway. This discrepancy may be not only due to the complicated interactions of protein kinase/phosphatase cascades, but also to cross-talk between the JA-, SA-, and ethylene-signalling pathways (see below). Since calcium-dependent protein kinases (CDPKs) are regularly involved in signal transduction of a variety of biotic and abiotic stresses (LUDWIG *et al.*, 2004), their involvement as active protein cascades in herbivore/wound responses cannot be excluded. CDPKs compose a large family of serine/threonine kinases in plants (for example, 34 members in *Arabidopsis*) (CHENG *et al.*, 2002; HARMON *et al.*, 2000). JA has been reported to affect CDPK transcript and activity in potato plants (ULLOA *et al.*, 2002).

A transgenic *Arabidopsis* line in which the expression level of phospholipase Dα (PLDα) is suppressed has been reported to show a decreased wound-induced accumulation of phosphatidic acid and JA, as well as JA-inducible transcript activation (WANG *et al.*, 2000). Curiously, this loss-of-function mutant also featured decreased expression of the gene coding lipoxygenase2 (LOX2) but not of any other gene involved in the octadecanoid pathway. LOX2 is one of the key enzymes for the wound-induced synthesis of JA in chloroplasts (BELL *et al.*, 1995). Therefore, PLD may specifically regulate LOX2 upstream of the octadecanoid pathway that leads to JA. In addition, the involve-

ment of phospholipase A (PLA), which mediates the release of linolenic acid from cell membranes and is inducible by wound stimuli, has been also proposed for tomato (*Lycopersicon esculentum*) and other species (NARVÁEZ-VÁSQUEZ *et al.*, 1999).

Altogether, early and secondary cell signalling for herbivore-induced plant responses comprise: (1) the reception of an extracellular signal(s) such as high- or low-molecular weight factors from the herbivore (e.g. FACs), (2) V_m depolarization and an intracellular calcium influx, (3) the activation of protein kinase/phosphatase cascades, and (4) the release of linolenic acid from the cell membrane and subsequent activation of the octadecanoid pathway which leads finally to the synthesis of JA and other oxylipins. However, multiple signals, signal circulation by, for example, protein phosphorylation/dephosphorylation, and feedback regulation of signalling and metabolic pathways are likely to complicate the understanding of the accurate mechanism.

2.3. Oxylipin, SA, and their antagonism

JA and SA function as signalling molecules, which mediate induced plant responses toward herbivory and pathogen infection, resulting in the activation of distinct sets of defence genes (MALECK & DIETRICH, 1999; REYMOND & FARMER, 1998; TURNER *et al.*, 2002). Besides free JA, also a conjugate with the amino acid isoleucine may be involved in signalling (KRUMM *et al.*, 1995; STASWICK & TIRYAKI, 2004), but the significance of such conjugates still remains to be established. Inhibiting the performance of JA or SA obviously renders plants more susceptible to herbivore damage and pathogen infection (MCCONN *et al.*, 1997; ROYO *et al.*, 1999; RYALS *et al.*, 1996; VIJAYAN *et al.*, 1998). JA and SA act antagonistically, and are both required for the induced response following herbivore feeding or pathogen attack (CIPOLLINI *et al.*, 2004; ENGELBERTH *et al.*, 2001; MORAN & THOMPSON, 2001; OZAWA *et al.*, 2000a; SPOEL *et al.*, 2003). For instance, the graduated increase of SA accumulation in Lima bean leaves treated with alamethicin (ALA), a potent fungal elicitor of plant volatile emission, interferes with steps in the biosynthetic pathway downstream of 12-oxophytodienoic acid (OPDA), thereby reducing the rapid accumulation of JA (~ 40 min) (ENGELBERTH *et al.*, 2001) and, finally, the biosynthesis and emission of all JA-linked volatiles. On the other hand, enhanced levels of OPDA selectively induce the emission of the C_{16}-tetranorditerpene 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) in Lima bean (KOCH *et al.*, 1999). Consequently, the treatment of Lima bean leaves with various amounts of JA and SA caused the emission of characteristic blends of volatiles including TMTT, which strongly attracted the carnivorous natural enemies of spider mites (DICKE *et al.*, 1990;

OZAWA *et al.*, 2000a; SHIMODA *et al.*, 2002). Hence, JA and SA pathways may vie with each other to control and coordinate induced defences, since the emission of characteristic blends of herbivore-induced volatiles from attacked plants strongly depends on the delicate balance between JA and SA (ENGELBERTH *et al.*, 2001). Alternatively, both pathways may play a role in discriminating insect biting from mechanical wounding and thus may be a kind of filter that prevents unnecessary defence activation.

Whether or not the recently discovered phytoprostanes, which result from non-enzymatic oxidative transformation of linolenic acid into prostaglandine-type octadecanoids (THOMA *et al.*, 2003), are involved in defence responses against herbivores remains to be established. At least, gene expression analysis of *Arabidopsis* cell cultures treated with the phytoprostanes PPB_1-I or -II revealed that both compounds triggered a massive detoxification and defence response covering the expression of several glutathione *S*-transferases, glycosyl transferases and putative ATP-binding cassette transporters (LOEFFLER *et al.*, 2005).

2.4. Synergy between JA and ethylene

Undoubtedly, JA is among the most important signalling molecules of herbivore-induced responses. However, the activation of JA formation alone cannot explain all changes following wounding or herbivory. For example, transgenic potato plants over-expressing an allene oxide synthase gene, which is involved in the JA formation, failed to express a PI gene (*Pin2*) constitutively, despite the fact that transgenic plants exhibited six- to twelve-fold higher levels of endogenous JA than did non-transgenic plants (HARMS *et al.*, 1995). This observation indicates that JA is not always involved in the signalling pathways of wound/herbivore responses and that other mediators may play a role in the respective signalling cascades. As described above, SA is an example of a signalling molecule that acts antagonistically towards JA. In addition, other signalling pathways may positively affect JA action. Ethylene is such a candidate, as it induces the expression of defence genes, and its synthesis is induced by wounding, herbivore feeding, and JA treatment (ARIMURA *et al.*, 2002; O'DONNELL *et al.*, 1996). Hints of a synergy between JA and ethylene in the herbivory- or wound-induced responses are: (1) plant defence genes are synergistically induced by ethylene and JA/MeJA in tobacco and in wounded tomato plants (O'DONNELL *et al.*, 1996; XU *et al.*, 1994), (2) treatment of maize plants with 1-methylcyclopropene (1-MCP) reduced the production of ethylene and volatile emission following *M. sexta* feeding (SCHMELZ *et al.*, 2003), and (3) treatment of maize plants and Lima bean leaves with ethylene or its precursor 1-aminocyclopropane-1-carboxylic

acid (ACC) significantly promoted volatile emission induced by JA (HORIUCHI *et al.*, 2001; SCHMELZ *et al.*, 2003). In addition, simultaneous application of JA and ACC to Lima bean leaves increased the attractiveness of the pre-treated Lima bean leaf for predatory mites (e.g. *Phytoseiulus persimilis*) as compared to leaves treated with JA alone (HORIUCHI *et al.*, 2001). All these results suggest that the cross-talk between JA and ethylene is required for both direct and indirect defence responses to herbivory. The application of ethylene and ethephon (an ethylene-releasing compound) together with the application of MeJA to tobacco plants suppressed the induction of nicotine accumulation yet hardly affected the emission of bergamotene, a volatile sesquiterpene (KAHL *et al.*, 2000). Moreover, the application of 1-MCP to tobacco plants treated with oral secretions of *M. sexta* larvae increased the induced accumulation of nicotine but reduced fitness-relevant plant parameters such as the rate of stalk elongation or lifetime capsule production (VOELCKEL *et al.*, 2001). In this case, the interplay between ethylene and JA may reduce the level of nicotine present in a plant, thus reducing its autotoxicity as well as the physiological costs of this compound. Moreover, ethylene has been reported to positively regulate the induction of allene oxide synthase, an enzyme which catalyzes the production of a precursor of OPDA in the octadecanoid pathway, in *Arabidopsis* and tomato (LAUDERT & WEILER, 1998; O'DONNELL *et al.*, 1996; SIVASANKAR *et al.*, 2000). Also, the antagonistic interaction between ethylene and JA on defence gene expression, resulting in resistance of *Arabidopsis* plants to herbivory, has been proposed (ROJO *et al.*, 2003). These mechanisms are different among different plant species (e.g. tomato and *Arabidopsis*) and the other conditions (ROJO *et al.*, 2003; WANG *et al.*, 2002).

Several other signalling molecules may function synergistically with JA and ethylene in plant defence responses. For example, in potato abscisic acid has been shown to increase JA levels following mechanical damage to leaf tissues (DAMMANN *et al.*, 1997), whereas abscisic acid did not regulate any defence-related genes by itself (BIRKENMEIER & RYAN, 1998). Among other plant hormones, auxin is a likely candidate, as it also has a negative effect on the JA pathway (LEÓN *et al.*, 2001). Furthermore, spermine is considered as an integral constituent of wound/herbivory responses, because (1) spermine activates WIPK and SIPK in tobacco leaves and mitochondrial dysfunction via a signalling pathway in which reactive oxygen species and a calcium influx are involved (TAKAHASHI *et al.*, 2003), and (2) the expression of a *S*-adenosylmethionine (SAM) decarboxylase gene involved in the polyamine synthesis is induced in Lima bean leaves infested with spider mites (ARIMURA *et al.*, 2002).

Considering that spider mite-infested Lima bean leaves do not accumulate endogenous spermine, this polyamine may, however, play a minor role in wound/herbivory responses. Alternatively, polyamines such as spermine may accumulate locally in intercellular spaces, as has been shown for tobacco leaves infected with tobacco mosaic virus (YAMAKAWA *et al.*, 1998). More evidence is still required to fully understand the role of polyamines in stress responses.

2.5. Transcription

A subset of *cis*- and *trans*-transcription elements, which are involved in the JA-ethylene- and the SA-signalling pathways, has been characterised. In several plant species, the GCC box (AGCCGCC), an ethylene-inducible element, has been found in the promoter region of ethylene-inducible defence genes (OHME-TAKAGI *et al.*, 2000). Four and six ethylene-responsive transcription factors (ERF) that specifically interact with the GCC box were characterised from tobacco and *Arabidopsis*, respectively (FUJIMOTO *et al.*, 2000; OHME-TAKAGI & SHINSHI, 1995; SOLANO *et al.*, 1998). ERF genes can respond to various extracellular stimuli, such as wounding, pathogen infection, and drought stresses, by changing of their transcriptional levels and have so far been described for higher plants but not for yeast and other fungi (FUJIMOTO *et al.*, 2000; OHME-TAKAGI *et al.*, 2000). In *Arabidopsis*, ERF1 and AtERF1, AtERF2, and AtERF5 are known as active elements of GCC-associated transcriptions, while AtERF3 and AtERF4 act as suppressive elements of these transcriptions (FUJIMOTO *et al.*, 2000). As they bind to the same target sequence, their transcriptional regulation is thought to depend on either the DNA binding affinity or on the relative abundance of active ERFs in the nucleus, or on both factors. Moreover, such GCC-associated transcripts have been shown to be coordinated not only by ethylene, but also by JA (BROWN *et al.*, 2003). Conversely, JA-inducible transcription factors ORCAs (the octadecanoid-derivative responsive *Catharanthus* AP2-domain) have been characterised as a JA-responsive APETALA2 (AP2)/ERF-domain transcription factor in *Catharanthus roseus* (MENKE *et al.*, 1999; VAN DER FITS & MEMELINK, 2000). The overexpression of *Orca3* constitutively increased the expression of genes involved in several metabolic pathways (tryptophan decarboxylase, 1-deoxy-D-xylulose-5-phosphate synthase and desacetoxyvindoline 4-hydroxylase) involved in terpenoids and indole alkaloid formations (VAN DER FITS & MEMELINK, 2000). Recently, a WRKY transcription factor was shown to bind to a W-box, with the latter consisting of two reversed TAGC repeats and located in the promoter region of the cotton (+)-δ-cadinene (sesquiterpene) synthase gene CAD1 (XU *et al.*, 2004). The expression of this transcription

factor was induced by JA and an elicitor derived from *Verticillium dahliae*. Direct/ indirect synergisms or antagonisms between multiple *trans*-factors are likely to regulate genes via signalling mediators such as JA, ethylene, and SA.

The recent development of high-throughput microarray technology allows the simultaneous and systematic monitoring of the expression pattern of an immense number of plant genes. Such analyses have shown comprehensive transcript profiles in plants responding to insect feeding, mechanical wounding, JA, H_2O_2, and plant volatiles (ARIMURA *et al.*, 2000b; HALITSCHKE *et al.*, 2003; QU *et al.*, 2004; REYMOND *et al.*, 2000, 2004). For instance, the transcription of several genes was induced in *Arabidopsis* following mechanical wounding and *Pieris rapae* feeding (REYMOND *et al.*, 2000). Curiously, one gene encoding a hevein-like protein was induced by *P. rapae*, but not by mechanical wounding. As described above, induced plant responses to mechanical wounding and feeding herbivores may differ due to varying degrees and types of damage and the presence or absence of salivary compounds.

2.6. Systemic response

It is well known that plants respond to herbivory and mechanical wounding not only at the site of damage, but also in remote, undamaged leaves. The best systems studied in this context are solanaceous species, such as tomato, for which an 18-amino-acid peptide called systemin was discovered and claimed to represent an intercellular signalling molecule (PEARCE *et al.*, 1991). Systemin is derived from a 200-aminoacid precursor prosystemin that has been originally discovered in tomato. Transgenic tomato plants that constitutively express antisense prosystemin RNA reduce wound-induced transcript accumulation of PIs in systemic leaves and are more susceptible to *M. sexta* attack than are wild-type plants (OROZCO-CÁRDENAS *et al.*, 1993). Furthermore, a 160-kDa plasma membrane-bound, leucine-rich repeat receptor kinase was recently characterised as a systemin receptor (SR160) from suspension cultures of tomato cells (SCHEER & RYAN, 2002). Surprisingly, this receptor is identical to tBRI1, a brassinosteroid receptor in tomato, and homologous to BRI1 in *Arabidopsis* (MONTOYA *et al.*, 2002; SZEKERES, 2003; WANG & HE, 2004). The dual function of SR160/tBRI1, which remains to be unequivocally established, is very unique, as this receptor has two ligands (systemin and brassinosteroid) and apparently functions in development and defence responses (WANG & HE, 2004). Additional analyses demonstrated that the overexpression of *SR160* in suspension-cultured tobacco cells yielded systemin-binding activity and a systemin-induced alkalinisation response in the cells. These results indicate that post-

receptional steps of the signal transduction are also present in tobacco, another member of the family Solanaceae. In tobacco, two 18-amino-acid hydroxyproline-rich glycoproteins (TobHypSys I and II), which are potential PI-inducers in a similar manner to systemin, have been discovered (PEARCE *et al.*, 2001a, b). These two peptides are derived from a 165-amino-acid precursor that does not exhibit any homology to prosystemin from tomato, but is analogous to peptide hormones from animals and yeast. Recently, this type of peptide was also discovered in tomato, raising the question of whether these peptides represent a general type of defence signal in the plant kingdom (PEARCE & RYAN, 2003).

Today, systemin is no longer considered as a long-distance signal. A systemin-insensitive tomato mutant (*spr1, suppressor of prosystemin-mediated responses1*), deficient in a signalling step that couples systemin perception to the subsequent activation of the octadecanoid pathway, showed that the peptide acts at or near the local site of wounding, increasing JA-synthesis above the threshold that is required for the systemic response in tomato (LEE & HOWE, 2003). According to these results, systemin is now thought to up-regulate the JA biosynthetic pathway to generate a long-distance signal at the local site (for example, JA or OPDA as the transmissible signals), whose recognition in distal leaves is linked to octadecanoid signalling. Moreover, it was also found that the rapid and transient activation of early genes in response to leaf excision or wounding was not affected in *spr1* plants, indicating the existence of a S*pr1*- and systemin-independent pathway for wound signalling (LEE & HOWE, 2003).

Herbivore-induced systemic responses can be observed not only in solanaceous species but also in many other plant families. Poplar trees (*Populus trichocarpa x deltoides*) are able to trigger the activation of gene expressions systemically in undamaged leaves far from those that had been damaged by the forest tent caterpillar (*Malacosoma disstria*) (ARIMURA *et al.*, 2004a). This systemic response revealed acropetal (upper) but not basipetal (lower) direction, but the transport mechanism remains obscure. A putative apoplastic lipid transfer protein DIR1 has been suggested as a mobile signal, mediating an interaction between a leaf of *Arabidopsis* infected with *Pseudomonas syringae* and distant, uninfected leaves (MALDONADO *et al.*, 2002). It was speculated that DIR1 could be a co-signal with other lipids, such as oxylipins (JA), phosphatidic acid, and *N*-acylethanolamines, which travel through the vascular system of the plant. However, actual evidence for a possible involvement of DIR1 in an herbivore-induced defence is still lacking. In addition, electrical signals have been noted and claimed to mediate long-distance interactions in wounded tomato seedlings (WIL-

DON *et al.*, 1992). Plants may have multiple systems that enable accurate long-distance signalling. Thus, JA itself can act as a systemic signal in tobacco, which is formed in the wounded leaves and travels to the undamaged distal leaves and roots where the expression of PI and the nicotine biosynthesis are induced, respectively (BALDWIN *et al.*, 1994; LI *et al.*, 2002).

3. Biosynthesis of herbivore-induced plant volatiles (HIPVs)

3.1. Volatile terpenoids

Volatile terpenoids which can be induced by herbivore-feeding comprise monoterpenes (C_{10}), sesquiterpenes (C_{15}) and homoterpenes (C_{11} or C_{16}). All terpenoids are synthesised through the condensation of isopentenyl diphosphate and its allylic isomer dimethylallyl diphosphate by catalysis of farnesyl diphosphate (FPP) synthase via the mevalonate pathway (cytsol/ endoplasmic reticulum) or geranyl diphosphate (GPP) and geranylgeranyl diphosphate via the methyld-erythritol-1-phosphate pathway in plastids (LICHTENTHALER, 1999; RODRÍGUEZ-CONCEPCIÓN & BORONAT, 2002) (Fig. 3). A large, structurally diverse number of terpenoids are yielded by a large family of terpene synthases (TPS) using GPP and FPP as substrates. In *Arabidopsis*, 32 genes including two gibberellin biosynthetic genes are putative members of the TPS family (AUBOURG *et al.*, 2002), some function as mono-TPSs and sesqui-TPSs (BOHLMANN *et al.*, 2000; CHEN *et al.*, 2004; CHEN *et al.*, 2003a; FÄLDT *et al.*, 2003). Terpenoid formation is generally assumed to be regulated on the transcript level of the TPS genes (ARIMURA *et al.*, 2004a; DUDAREVA *et al.*, 2003; MCKAY *et al.*, 2003; SHARON-ASA *et al.*, 2003).

However, the regulation mechanism seems to be rather complex, because herbivore-induced TPS transcripts and terpene emissions are affected by several factors (for example, by diurnal rhythmicity and distance to herbivore-damaged tissue) (ARIMURA *et al.*, 2004b; MILLER *et al.*, 2005). Figure 4 shows temporal patterns of volatile emissions in Lima bean leaves following herbivore attack by *S. littoralis* over 4 days. The release of terpenoids and the C_6-volatile (3*Z*)-hex-3-enyl acetate follows diurnal cycles with increased emissions during the light period and reduced emissions during darkness. This result is in line with findings in Lima beans treated with ALA and poplar leaves infested with forest tent caterpillars, where the volatile emissions or the TPS expressions follows diurnal cycles (ARIMURA *et al.*, 2004a; KUNERT *et al.*, 2002). In this context it would be interesting to study to what extent volatile emissions are linked to the endogenous biological clock.

On the other hand, a single event of mechanical damage or the application of ALA to *Lotus japonicus* plants was not

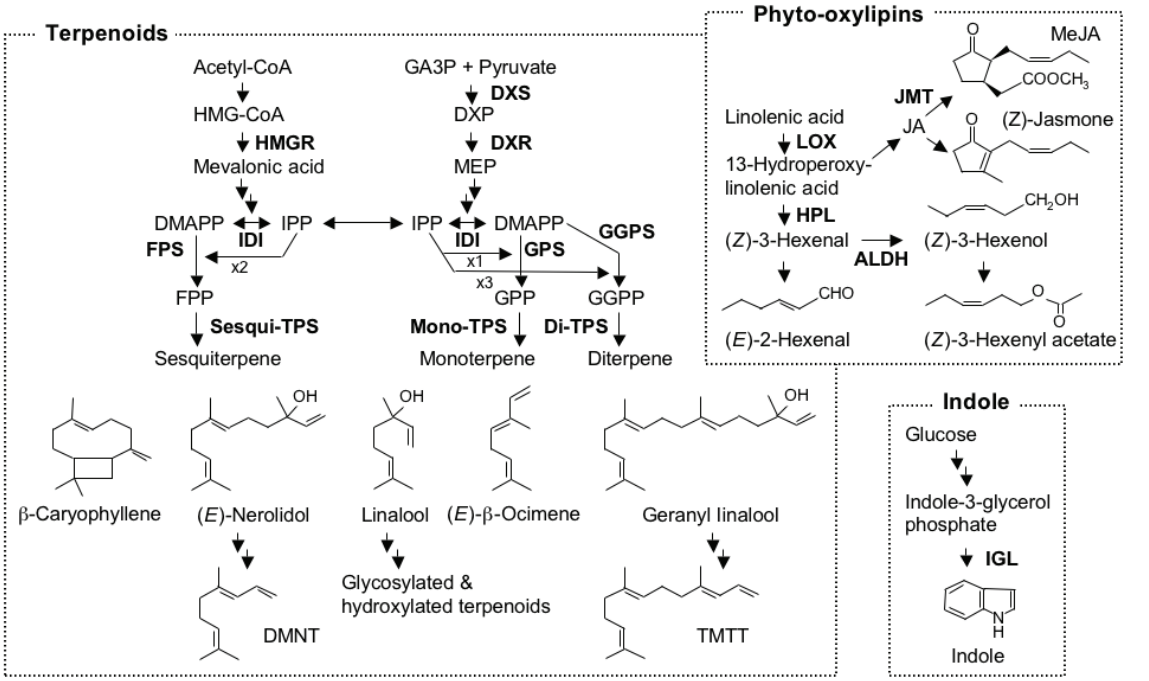


Figure 3. Biosynthetic pathways required for herbivore-induced plant volatiles. Elements in bold are enzymes. Abbreviations: ALDH, aldehyde dehydrogenase; DMAPP, dimethylallyl diphosphate; DMNT, (*E*)-4,8-dimethyl-1,3,7-nonatriene; DXP, 1-deoxy-D-xylulose-5-phosphate; DXR, DXP reductoisomerase; DXS, DXP synthase; FPP, farnesyl diphosphate; FPS, FPP synthase; GGPP, geranylgeranyl diphosphate; GGPS, GGPP synthase; GPP; geranyl diphosphate; GPS, GPP synthase; HMG-CoA, 3-hydroxy-3-methyl-glutaryl CoA; HMGR, HMG-CoA reductase; HPL, fatty acid hydroperoxide lyase; IDI, IPP isomerase; IGL, indole-3-glycerol phosphate lyase; IPP, isopentenyl diphosphate; JA, jasmonic acid; JMT, JA carboxyl methyl transferase; LOX, lipoxygenase; MeJA, methyl jasmonate; MEP, 2-*C*-methyl-D-erythritol-4-phosphate; TMTT, 4,8,12-trimethyltrideca-1,3,7,11-tetraene; TPS, terpene synthase.

shown to result in an increased release of (*E*)-β-ocimene from the plants, despite increased transcript levels of a (*E*)-β-ocimene synthase (*LjEβOS*) gene (ARIMURA *et al.*, 2004b). Furthermore, transgenic *Petunia hybrida* plants that overexpressed *Clarkia breweri S*-linalool synthase (lis) produced minor amounts of volatile *S*-linalool, but conjugation to the non-volatile *S*-linalyl-β-D-glucopyranoside which is absent in wild-type *C. breweri* (LÜCKER *et al.*, 2001). Similarly, transgenic *Arabidopsis* plants overexpressing strawberry linalool/nerolidol synthase (NaNES1) produced not only volatile linalool but also its glycosylated and hydroxylated derivatives (AHARONI *et al.*, 2003). Several volatile terpenoids such as linalool can be glucosylated or hydroxylated by the catalysis of glucosyl transferase and cytochrome P450 monooxygenases (P450), respectively. The biosynthesis of two tetranorterpenoids (homoterpenes), (*E*)-4,8-dimethyl-1,3,7-nonatri-

ene (DMNT) and TMTT, has been proposed to proceed via an oxidative degradation by P450 enzymes of the sesquiterpene (*E*)-nerolidol and the diterpene geranyl linalool as precursors (DONATH & BOLAND, 1995). Herbivore-induced formation of DMNT is regulated at the level of both a (*E*)-nerolidol synthase and a P450-catalysed process in maize (DEGENHARDT & GERSHENZON, 2000). Hence, multiple mechanisms including TPS transcripts and enzymatic modifications seem to contribute to the control of terpenoids synthesis.

Terpenoid compounds emitted from Lima bean plants infested with spider mites act as airborne signals, activating JA/ethylene-induced defence responses and enhancing the resistance against spider mites in the leaves of neighbouring conspecific plants (ARIMURA *et al.*, 2000a, 2002). The signalling pathway involved in the inter-plant communication is mediated by calcium influxes into cells and protein-phosphorylation and dephosphorylation in the receiver plants. Similarly, the non-volatile C_{20} diterpene (11*E*,13*E*)-labda-11,13-diene-8α,15-diol (WAF-1) activates WIPK, SIPK, other MAPK and enhanced transcript levels of wound- and pathogen-inducible genes in tobacco (SEO *et al.*, 2003). The treatment of the tobacco leaves with WAF-1 enhanced resistance to the tobacco mosaic virus infection. Thus, both volatile and non-volatile terpenoids can mediate between-

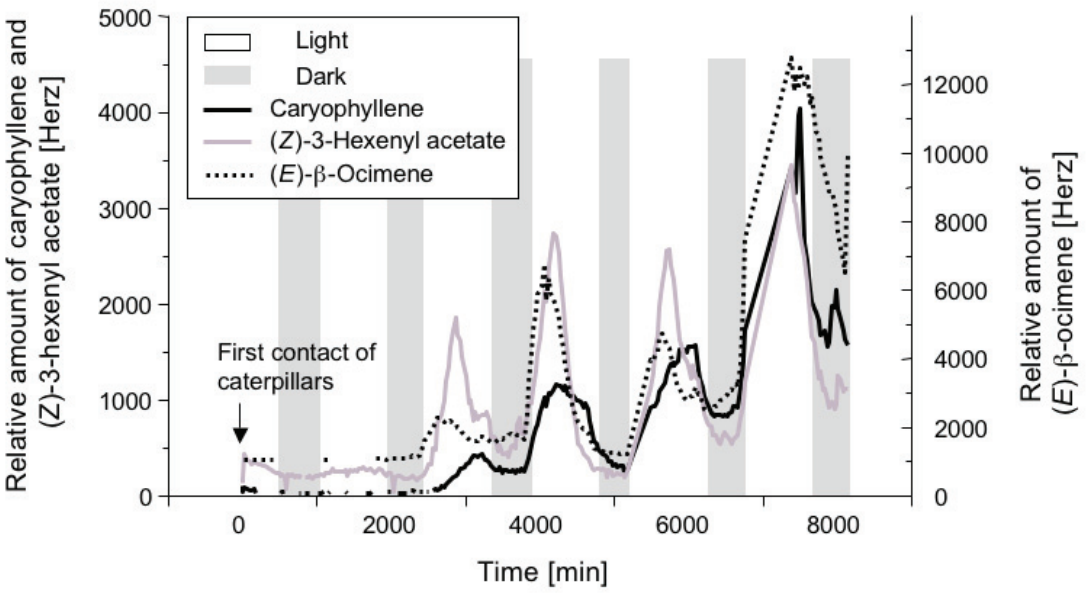


Figure 4. HIPV emission from Lima bean leaves during insect feeding. Volatile emissions released from five fully developed (2-week-old) plantlets infested with twelve *Spodoptera littoralis* larvae (third instar) were continuously monitored using an automated gas chromatographic system equipped with a preconcentration unit (zNose™) (KUNERT *et al.*, 2002).

cell and inter-plant signalling of induced plant defences.

3.2. C_6-volatiles

Six-carbon (C_6)-volatiles or 'green leaf volatiles', as they are also called, when they are released from plant leaves immediately after wounding, include isomers of hexenol, hexenal, and hexenyl acetate (HATANAKA, 1993). Amazingly, the formation of (3*Z*)-hex-3-enal, an initially formed C_6-volatile, can be observed within 20 s after the disruption of *Arabidopsis* leaf tissues (MATSUI *et al.*, 2000). This formation is probably due to an array of enzymatic activations (phospholipase, LOX, and fatty acid hydroperoxide lyase [HPL]) and/or the encounter of these enzymes with their substrates α-linolenic acid, linoleic acid or 13-hydroperoxide (MATSUI *et al.*, 2000). Pre-treatment of the *Arabidopsis* leaves with quinacrine, a lipase inhibitor, suppressed the formation of (3*Z*)-hex-3-enal but increased the accumulation of α-linolenic acid. These results apparently indicate that the cleavage of free unsaturated fatty acids from membrane lipids is the rate-limiting step for the rapid formation of hexenal. The aldehyde isomers (3*Z*)-hex-3-enal and (2*E*)-hex-2-enal contain an α,β-unsaturated carbonyl group, known as reactive electrophile (ALMÉRAS *et al.*, 2003), which induces the emission of volatile terpenoids in tomato plants (FARAG & PARE, 2002), the accumulation of endogenous JA as well as the expression of defence genes in several other plant species (ARIMURA *et al.*, 2001; BATE & ROTHSTEIN, 1998; ENGELBERTH *et al.*, 2004). It has also been reported that both C_6-aldehydes and -alcohols reduce tobacco aphid fecundity on tobacco leaves (HILDEBRAND *et al.*, 1993). Moreover, antisense-mediated HPL depletion significantly reduced the levels of hexanal and hexenal and increased the aphid fecundity on potato plants (VANCANNEYT *et al.*, 2001), suggesting that C_6-volatiles play a role as either direct plant defence agents or signalling molecules which elicit defence reactions.

However, C_6-volatiles may not always benefit plants. (2*E*)-Hex-2-enal inhibits the germination and subsequent growth of soybeans as well as the germination of apple pollen (GARDNER & AGRAWAL, 2002; HAMILTON-KEMP *et al.*, 1991). Furthermore, (2*E*)-hex-2-enal reduces the germination frequency of the MeJA-insensitive *Arabidopsis* mutant *jar1-1*, suggesting that (2*E*)-hex-2-enal and MeJA are recognized in plants by different mechanisms (BATE & ROTHSTEIN, 1998). A rapid formation of C_6-volatiles after wounding not only serves as protection against herbivores or pathogens, but may also be toxic for the plant itself. This effect could be interpreted as some kind of strategic retreat, as has been observed for hypersensitive cell death in response to pathogen infection. It remains to be investigated whether the transient burst of

wound-induced C_6-volatiles is detrimental for the growth and development of a plant in the long term.

In contrast to C_6-aldehydes and -alcohols, the emission of (3*Z*)-hex-3-enyl acetate can frequently be observed a few hours after the first impact of herbivore feeding or mechanical damage (ARIMURA *et al.*, 2000a; RÖSE & TUMLINSON, 2004; TURLINGS *et al.*, 1995), as seen in the emission of herbivore-induced terpenoids (Fig. 2). Since the emission of both (3*Z*)-hex-3-enyl acetate and β-ocimene can be induced by JA and ethylene (HORIUCHI *et al.*, 2001), the signalling pathway may be identical. However, nothing is known about whether or not the biological function of hexenyl acetate differs from the two early C_6-volatiles.

3.3. Volatile phytohormones and indole

Like JA, MeJA is also considered one of the most important signalling molecules of herbivore-induced plant defence. An early study showed that synthesis of PI proteins in the leaves of solanaceous species is induced by exogenous application of MeJA or natural emission of MeJA from sagebrush plants (*Artemisia tridentata*) (FARMER & RYAN, 1990). Transgenic *Arabidopsis* overexpressing JA carboxyl methyl transferase (JMT), an enzyme that methylates JA to form MeJA, showed a three-fold increase of the endogenous MeJA level without altering JA levels (SEO *et al.*, 2001). Furthermore, transgenic plants exhibited constitutive transcript accumulation of JA-responsible genes and enhanced resistance against the virulent fungus *Botrytis cinere* (SEO *et al.*, 2001). These results indicate the importance of MeJA as a cellular signal regulator and potential inter-cellular, and intra- and inter-plant signal transducers. The other JA derivative, (*Z*)-jasmone, has been found to repel the lettuce aphid (*Nasonovia ribisnigri*) and to induce (*E*)-β-ocimene, resulting in an increased attractivity of the plants to the aphid parasitoid *Aphidius ervi* (BIRKETT *et al.*, 2000). However, it has been also reported that MeJA and (*Z*)-jasmone are only moderately induced in *Nicotiana attenuata* plants in response to feeding herbivores (9% of the JA accumulation) (VON DAHL & BALDWIN, 2004). Functionally, these JA derivatives may be of minor importance for defence and signalling. Otherwise, the importance may be limited in a few plant species such as *A. tridentata*.

The *Arabidopsis* genome contains 24 genes, which belong to a structurally related group of methyl transferases (D'AURIA *et al.*, 2003). These have been classified as one gene family termed AtSABATH, of which JMT is a member. AtBSMT1, another member of this family, has recently been characterised as SA carboxyl methyl transferase, an enzyme that methylates SA to form MeSA (CHEN *et al.*, 2003b). The transcript accumulation of AtBSMT1 can be induced by any of the

following: the fungal elicitor ALA; feeding *Plutella xylostella*; uprooting; mechanical wounding; and exogenous application of MeJA to *Arabidopsis* leaves. In some of these cases, the emission of MeSA is also induced. Besides its role as HIPV in attracting carnivores, such as the mites *P. persimilis* and *Amblyseius potentillae* (DICKE *et al.*, 1990c), MeSA can also induce defence responses in plants (SHULAEV *et al.*, 1997).

Occasionally indole has been observed as a minor constituent of herbivore-induced volatile blends. In maize plants, a gene has been characterised as indole-3-glycerol phosphate (IGP) lyase, which catalyses the formation of free indole from IGP (FREY *et al.*, 2000). Both volicitin and MeJA were shown as selective inducers of the gene expression and the indole emission from maize plants (FREY *et al.*, 2000, 2004).

4. Ecology of induced indirect defences

4.1. Variability of herbivore-induced plant volatiles

The emission of volatile organic compounds seems to be very common across the plant kingdom and is not limited to certain life forms (see (DICKE & VET, 1999) for a compilation of species). Odour blends emitted by herbivore-infested plants are complex mixtures, often composed of more than 100 different compounds, many of which occur as minor constituents (DICKE, 1999b). Different plant species vary in the headspace composition (BARATA *et al.*, 2000; TURLINGS *et al.*, 1993) yet also share compounds, for example, (*E*)-β-ocimene, (3*Z*)-hex-3-enyl acetate, and DMNT in Lima beans and cucumber (TAKABAYASHI *et al.*, 1991). Even within a species, the volatile blends emitted upon herbivore damage differ both quantitatively and qualitatively depending on the genotype or cultivar (GOUINGUENE *et al.*, 2001; KRIPS *et al.*, 2001; SCUTAREANU *et al.*, 2003), the leaf developmental stage (TAKABAYASHI *et al.*, 1994b), or the plant tissue attacked by an herbivore (TURLINGS *et al.*, 1993), as well as on abiotic conditions (light intensity, time of year, water stress) (TAKABAYASHI *et al.*, 1994a). Beyond that, the time of the day also influences the composition of the emitted volatile blend (Fig. 4): for example, *Nicotiana tabacum* releases several HIPVs exclusively at night. These nocturnally emitted compounds were repellent to female moths (*Heliothis virescens*), which search for oviposition sites during the night (DE MORAES *et al.*, 2001). Moreover, the blend composition strongly depends on the type of damage (e.g. herbivore feeding versus oviposition (HILKER & MEINERS, 2002)) inflicted upon a given plant individual as well as on the time elapsed after leaf damage (Fig. 4). To all the variability that already exists on the plant level, several studies have added that the emitted blends also vary

with the herbivore species (DICKE, 1999a), and even different ontogenetic stages of the same herbivore (GOUINGUENE *et al.*, 2003; TAKABAYASHI *et al.*, 1995) may influence the headspace composition. However, differences between volatile blends seem to be largest among different plant species and smallest among plants of one species infested by different herbivores (DICKE, 1999a; TAKABAYASHI *et al.*, 1991).

The high variability that characterises the chemical composition of emitted blends raises the question of the ecological relevance of these differences and whether arthropods can discriminate among these complex mixtures. Electroantennogram (EAG) analyses allow individual blend components to be differentiated in order to determine which compounds or combination of different compounds can be perceived by an arthropod (DU *et al.*, 1998; WEISSBECKER *et al.*, 2000). Further information on the physiological perception of different plant odours by arthropods have been obtained from in vivo calcium-imaging measurements that provide additional information on how the olfactory information is processed in the arthropod brain (GALIZIA *et al.*, 2000; SKIRI *et al.*, 2004). However, only the additional inclusion of behavioural assays, in which the arthropods are simultaneously exposed to two or more odours among which they have to choose, provides information on whether an odour has an attracting, repelling, or neutral effect on the behaviour of the focal arthropod.

4.2. Specificity and behavioural responses to HIPVs

Much has been learned from such behavioural assays on the ability of arthropods to discriminate different odour blends. As the production of HIPVs has been originally described in the context of host-location cues for parasitic wasps (TURLINGS *et al.*, 1990), most researchers have focussed on the role of plant-derived volatiles in determining the ability of different parasitoid or carnivore species to detect their herbivorous hosts or preys. Several studies of an increasing number of tritrophic plant-herbivore-carnivore systems have indicated that the ability of the carnivores and parasitoids to discriminate different odour blends depends very much on their degree of dietary specialization (VET & DICKE, 1992), and on their level of deprivation, as well as on their previous experience (DICKE, 1999a). The particular behavioural response - that is whether an herbivore or carnivore is attracted or repelled, or reacts neutrally to a specific blend of HIPVs - seems to depend strongly on the level of plant induction (GOLS *et al.*, 2003; HEIL, 2004b; HORIUCHI *et al.*, 2003b).

However, plants do not only interact with other organisms above ground. Recently, evidence has shown for the first time that mechanisms similar to the ones

described above- may also work below-ground: Entomopathogenic nematodes (*Heterohabditis megidis*) are attracted as yet unidentified chemicals that are released from roots of a coniferous plant (*Thuja occidentalis*) when weevil larvae (*Otiorhynchus sulcatus*) attack (VAN TOL *et al.*, 2001). Moreover, systemically released HIPVs have been shown to attract the specialist parasitoids of root-feeding larvae (NEVEU *et al.*, 2002). Although currently multitrophic below-ground interactions and the links between multitrophic above- and below-ground interactions are poorly understood, future research will undoubtedly address these questions (VAN DER PUTTEN *et al.*, 2001).

4.3. Ecological function of HIPVs in nature

Most studies regarding HIPV emission have been conducted under artificial greenhouse or laboratory conditions. Facing the above-mentioned variability, the question arises whether plants benefit from the emission of HIPVs under natural growing conditions. To date, few studies exist that show predators are attracted to herbivore-infested plants under field conditions (DRUKKER *et al.*, 1995; DU *et al.*, 1998; SCUTAREANU *et al.*, 1997; SHIMODA *et al.*, 1997). Moreover, specialist parasitoid wasps were able to distinguish among maize, cotton, and tobacco plants that were infested by their herbivorous hosts from those which were under non-host attack (DE MORAES *et al.*, 1998). Caterpillars (*S. exigua*) placed on tomato plants (*L. esculentum* var. *Ace*) that had been grown in an agricultural system and induced previously with exogenously applied JA, suffered from higher parasitism rates by an endoparasitic wasp (*Hyposoter exiguae*) than control plants (THALER, 1999). A further field study of native tobacco (*N. attenuata*) demonstrated that, indeed, the release of volatiles increased the predation rates of tobacco hornworm eggs by a generalist predator (*Geocoris pallens*) (KESSLER & BALDWIN, 2001). Recently, Lima bean plants grown in their natural environment, followed by repeated treatments with JA solutions for 5 weeks were shown to respond with an increased seed set (HEIL, 2004a). Since both the emission of HIPV as well as the secretion of extrafloral nectar had been up-regulated by this treatment, it remained unclear which indirect defence was responsible for the observed beneficial effect. More field trials are clearly necessary to determine the ecological relevance of individual factors and to verify results that have been gathered in laboratory or greenhouse experiments.

4.4. Extrafloral nectar

In addition to releasing induced volatiles, some plant species have developed other ways to attract predatory arthropods that will defend them. These include both the provision of shelter in so-called leaf domatia (AGRAWAL & KARBAN, 1997), and

the offer of attractive food sources such as food bodies or extrafloral nectar (EFN). Extrafloral (EF) nectaries are specialized nectar-secreting organs that may occur on virtually all vegetative and reproductive plant structures, yet do not involve pollination (ELIAS, 1983). They have been described for approximately a thousand plant species ranging over 93 plant families (KOPTUR, 1992) of flowering plants and ferns, but they are absent in gymnosperms (ELIAS, 1983). EFNs are aqueous solutions that comprise mainly sucrose, glucose, and fructose, but other sugars, amino acids and other organic compounds may be present in some species (BENTLEY, 1977a). The secreted sugars are mainly derived from the phloem (FAHN, 2000; FREY-WYSSLING, 1955) or synthesised in the region of the nectary (BARGONI, 1972). EF nectaries secrete small amounts of nectar throughout the day. The secretion of nectar can follow circadian rhythms (ELIAS, 1972; HEIL *et al.*, 2000b; PASCAL & BELIN-DEPOUX, 1991; RAINE *et al.*, 2002) or be relatively constant throughout day and night (BENTLEY, 1976, 1977b).

The mechanisms of nectar secretion are poorly understood: Some researchers have described secretion as a passive process, whereas others discuss it as an elimination of surplus sugars (BENTLEY, 1977a). However, there is evidence that nectar secretion is an active secretory process: It requires the expenditure of metabolic energy (LÜTTGE, 1971), and EF nectaries strongly resemble secretory cells which contain large numbers of mitochondria and show dense protoplasts and large nuclei (BENTLEY, 1977a). Furthermore, the secretion of EFN can be induced by JA treatment. Originally discovered in *Macaranga tanarius* (HEIL *et al.*, 2001), nectar secretion is suggested to be inducible by leaf damage. This seems to be a widespread phenomenon (HEIL, 2004a; NESS, 2003; WÄCKERS & BEZEMER, 2003; WÄCKERS *et al.*, 2001), which may be linked to the same or similar signalling pathways as those discussed for the induction of volatile biosynthesis. Inhibitors that suppress the release of linolenic acid or interfere with the production of linolenic acid hydroperoxides completely suppress the damage-induced flow of EFN and, hence, clearly demonstrate the involvement of oxylipin-based signalling in EFN-induction. Interestingly, also the attack of a below-ground herbivore (*Agriotes lineatus*) on cotton plants (*Gossypium herbaceum*) induced above-ground production of EFN (WÄCKERS & BEZEMER, 2003). In *Vicia faba*, even the number of EF nectaries increased following leaf damage (MONDOR & ADDICOTT, 2003).

Generally, the secretion of EFN is believed to function as an indirect anti-herbivore defence by attracting and hence increasing the presence of putative plant defenders on nectar-secreting plant parts.

Most studies focussed on the protective effect of ants on entire plants or individual plant parts, demonstrating a beneficial function of these insects (BENTLEY, 1977a; KOPTUR, 1992). However, contradictory observations that detected no measurable preventive effect of EFN-attracted ants also exist (BECERRA & VENABLE, 1989; BOECKLEN, 1984; MODY & LINSENMAIR, 2004; O'DOWD & CATCHPOLE, 1983; RASHBROOK *et al.*, 1992). In these cases, the lack of protection could be explained by, for example, (1) differences in the aggressiveness of the attracted ant species (DEJEAN *et al.*, 2000; HORVITZ & SCHEMSKE, 1984; MODY & LINSENMAIR, 2004), (2) differences in foraging behaviour of ant species from different habitats (BARTON, 1986; INOUYE & TAYLOR, 1979), and (3) a varying susceptibility of the herbivores to ant predation (FOWLER & MACGARVIN, 1985; HEADS & LAWTON, 1985). Besides ants, the EF nectaries attract a diverse spectrum of other arthropods including Araneae, Diptera, Coleoptera, and Hymenoptera (see (KOPTUR, 1992) for review). Due to their predatory or parasitoid ways of life, many of these non-ants, such as ichneumonid, braconid and chalcid wasps (BUGG *et al.*, 1989; KOST & HEIL, 2005a; STAPEL *et al.*, 1997), jumping spiders (Salticidae) (RUHREN & HANDEL, 1999), phytoseiid mites (VAN RIJN & TANIGOSHI, 1999) or tachinid flies (PEMBERTON & LEE, 1996), can as well reduce the number of herbivores. Both ants and wasps exerted beneficial effects on the EF nectary-bearing plant *Turnera ulmifolia* when selectively excluded, yet no additive increase was observed when both insect groups had access (CUAUTLE & RICO-GRAY, 2003).

The emission of floral odours by plants is very important for attracting pollinators to their floral nectars. Some of these floral nectars also scent by themselves (RAGUSO, 2004), whereas nothing is known about the headspace of EFNs. Beyond these odours, which communicate the location, abundance, and quality of these nectars to higher trophic levels, other mechanisms may help to guide EFN-feeding arthropods to remote nectar sources. First, some EF nectaries are coloured providing visual cues for foraging arthropods (KOPTUR, 1992). Additionally, increased amounts of both HIPVs and EFN (Table 1) would allow foraging arthropods to use the emitted volatile organic compounds as a cue to detect nectar sources from longer distances. Future research should try to disentangle and quantify the costs and benefits of one of these indirect defences for a plant and figure out to what extent each of them contributes to plant defence in nature.

4.5. Evolution of HIPVs and EFN

The evolutionary origin of HIPV emission and EFN secretion remains unknown. As described above, C_6-volatiles or terpe-

Table 1 Compilation of species which both emit HIPVs and feature extrafloral nectaries. Additional information on the inducibility is presented (for abbreviations see below).

Family	Species	Nectar	inducible	HIPVs	inducible	Reference
Euphorbiaceae	*Manihot esculenta*	n.i.	n.i.	+	H 1	GARY & FOSTER, 2004; GNANVOSSOU *et al.*, 2001
Leguminosae	*Phaseolus lunatus*	+	JA	+	JA	HEIL, 2004a
	Vicia faba	n.i.	n.i.	+	H 2	COLAZZA *et al.*, 2004; FIGIER, 1971
	Vignia unguiculata	n.i.	n.i.	+	H3	KUO & PATE, 1985; VAN DEN BOOM *et al.*, 2004
Malvaceae	*Gossypium herbaceum*	+	M, H 4, 5	+	H 5, 6	BEZEMER *et al.*, 2004; WÄCKERS & BEZEMER, 2003; WÄCKERS *et al.*, 2001
	Gossypium hirsutum	n.i.	n.i.	+	H 6 , 7, 8	LANDOLT, 1993; LOUGHRIN *et al.*, 1995; MCCALL *et al.*, 1994; STAPEL *et al.*, 1997
Rosaceae	*Prunus serotina*	n.i.	n.i.	+	H 9	RUTHER *et al.*, 2002; TILMAN, 1978
Salicaceae	*Populus deltoides*	n.i.	n.i.	+	H 10	ARIMURA *et al.*, 2004a; CURTIS & LERSTEN, 1974
	Populus trichocarpa	n.i.	n.i.	+	H 10	ARIMURA *et al.*, 2004a; TRELEASE, 1881
Solanacea	*Solanum nigrum*	n.i.	n.i.	+	H 11, 12	KEELER, 1979; SCHMIDT *et al.*, 2004

M mechanical damage, **H 1** Spider mite (*Mononychellus tanajoa* BONDAR), **H 2** Pentatomid bug (*Nezara viridula* LINNAEUS), **H 3** Spider mite (*Tetranychus urticae* KOCH), **H 4** Mediterranean Brocade (*Spodoptera littoralis* BOISDUVAL), **H 5** Wireworms (*Agriotes lineatus* LINNAEUS), **H 6** Beet armyworm (*Spodoptera exigua* HÜBNER), **H 7** Tobacco budworm (*Heliothis virescens* FABRICIUS), **H 8** Corn earworm (*Helicoverpa zea* BODDIE), **H 9** Forest cockchafer (*Melolontha hippocastani* FABRICIUS), **H 10** Forest tent caterpillar (*Malacosoma disstria* HÜBNER), **H 11** Colorado potato beetle (*Leptinotarsa decemlineata* SAY), **H 12** Death's Head Hawkmoth (*Acherontia atropos* LINNAEUS).

noids may repel herbivores (AVÉ *et al.*, 1987; VANCANNEYT *et al.*, 2001), be toxic for phytophages (CRANKSHAW & LANGENHEIM, 1981; HOBALLAH *et al.*, 2004), or act anti-microbially (CROFT *et al.*, 1993). HIPVs are therefore discussed as having originally functioned as a direct defence against pathogens or herbivores (DICKE, 1999c). However, in this case, non-volatile defensive compounds rather than volatile substances should have evolved to fulfil this purpose, as they would not diffuse so easily in the environment (JANSSEN *et al.*, 2002). HIPV emission is an active process that does not necessarily depend on cell damage at the

site of emission (DICKE & VAN LOON, 2000). Even if the first volatile release by an herbivore-damaged plant was incidental, natural enemies might have been immediately able to use this cue to locate the responsible herbivores; hence, natural selection should operate on the plant to optimize these signals (JANSSEN *et al.*, 2002).

The evolution of EF nectaries is considered a very simple process (BENTLEY, 1977a) that may have occurred several times independently (BENTLEY, 1977a; ELIAS, 1983). This development has resulted in an extreme variety of plant taxa that feature a wide structural diversity of EF nectaries (ZIMMERMANN, 1932); yet these structures may be absent in one of two very closely related species (BENTLEY, 1977a). Recently, a 2-year study has shown that traits of EF nectaries were heritable and that casual ant associates acted as agents of selection on these traits (RUDGERS, 2004). Even if corresponding studies on other EF nectary-bearing plants or plants that feature HIPVs are missing, these two indirect defences seem to be evolutionarily plastic and to remain stable due to strong selection pressure. However, the genetic basis of these two traits has been investigated only for the development of EF nectaries in cultivated cotton (MEYER & MEYER, 1961; RHYNE, 1965) and *Acacia koa* (DAEHLER *et al.*, 1999): For cotton crossing, studies showed that the inheritance of “nectaryless” was transferred by two pairs of recessive genes (i.e. *ne-1* and *ne-2*) (MEYER & MEYER, 1961; RHYNE, 1965).

In addition to the benefits discussed above, the concept of fitness costs provides a powerful explanation for the evolution of induced plants defences (CIPOLLINI *et al.*, 2003; HEIL, 2002): Costs cover any trade-off between resistance and other fitness-relevant processes (HEIL, 2002). Inducibility itself (i.e. the ability to change per se) can raise fitness costs by, for example, increasing the lag time until the defence is up-regulated, during which the plants remains susceptible to herbivores (DEWITT *et al.*, 1998; ZANGERL, 2003). Further evolutionarily relevant costs comprise allocation costs (HERMS & MATTSON, 1992), genetic costs (AGRAWAL *et al.*, 2002a; MITCHELL-OLDS *et al.*, 1996), autotoxicity costs (BALDWIN & CALLAHAN, 1993), opportunity costs (BALDWIN & HAMILTON, 2000), and ecological costs (Fig. 5) (AGRAWAL *et al.*, 2002b; PRICE *et al.*, 1980). Inducible defence strategies are generally assumed to reduce the fitness of a plant when expressed under enemy-free conditions (HAUKIOJA & HAKALA, 1975) as compared to costs of a constitutive (i.e. permanent) expression. The physiological expenditures of EFN production that could cause allocation trade-offs within the plant are assumed to be very low, yet this has rarely been tested: For the Balsa tree *Ochroma pyramidale*, the physiological expenses of

EFN secretion have been calculated to account for 1 % of the total energy investment per leaf (O'DOWD, 1979). In contrast, a phylogenetical analysis of American *Acacia* species revealed that constitutive EFN secretion was found to be the derived state which developed as an adaptation to obligate myrmecophytism. This may indicate that a constitutive flow of EFN is costly and only profitable when rewarded by permanent ant-protection (HEIL *et al.*, 2004b). The actual costs probably depend on the level of EFN excretion and physiological limitations such as the availability of water or nutrients. EFF secretion even ceases in the absence of nectar feeders, yet this may be a passive process and not necessarily a plant-regulated strategy to save costs (HEIL *et al.*, 2000b). The physiological expenditures of HIPV are expected to be relatively high (GERSHENZON, 1994a, b; GULMON & MOONEY, 1986), yet predictions remain controversial (DICKE & SABELIS, 1989; GODFRAY, 1995; SABELIS & DE JONG, 1988) and experimental evidence is rare. In maize plants, the first hints of allocation costs for the production of HIPVs have been seen (HOBALLAH *et al.*, 2004). However, to what extent simultaneously induced direct defences accounted for the measured costs remained unknown. Interestingly, these allocation costs were detectable only in young plants, with compensation for their metabolic investment occurring during maturation. Finite resource availabilities for plants may also lead to trade-offs between direct and indirect defences (HEIL *et al.*, 2002; RUDGERS *et al.*, 2004); these seem to be more common in obligate ant–plant mutualisms (HEIL *et al.*, 1999, 2000a; KOPTUR, 1985) than in facultative associations (RUDGERS *et al.*, 2004).

Both EFN and HIPVs are openly presented cues that may non-specifically attract members of higher trophic levels. Such loose forms of facultative mutualisms are especially prone to exploitation by undesired arthropods. For example, the attraction of non-beneficial or even herbivorous species feeding on EFN results in costs rather than benefits for the respective plant (Fig. 5) (DEVRIES & BAKER, 1989; HEIL *et al.*, 2004a; KOPTUR & LAWTON, 1988). HIPVs emitted from strongly induced plants denote not only parasitoids or predators, the presence of potential prey organisms but, additionally, a hint at increased numbers of competitors (JANSSEN *et al.*, 1997, 1995a, b). Furthermore, by calling on strongly induced plants, carnivores or parasitoids may well run the risk of falling prey themselves to a hyperparasite or a predator (HÖLLER *et al.*, 1994). The resulting deterrent effect of HIPVs on beneficial arthropods represents an ecologically relevant fitness cost for the herbivore-attacked plant (Fig. 5).

Signals emitted from infested plants can also provide information to other trophic levels (Fig. 5): Conspecific herbi-

vores, for example, can be attracted to infested plants (BERNASCONI *et al.*, 1998; BOLTER *et al.*, 1997; HORIUCHI *et al.*, 2003b), because this cue denotes the location of palatable host plants (INUI *et al.*, 2003) and suitable oviposition sites (STANJEK *et al.*, 1997) as well as the presence of potential mating partners (RUTHER *et al.*, 2002). Such a 'miscue' from the plant's perspective results as well in ecological fitness costs for the plant. On the other hand, conspecific or heterospecific herbivores may be deterred by the emitted volatiles (BERNASCONI *et al.*, 1998; DE MORAES *et al.*, 2001), as strongly induced plants may point to competition with conspecific or heterospecific herbivores or to an enemy-dense patch (HORIUCHI *et al.*, 2003a). In this case, the release of HIPVs would act as a direct defence (DE MORAES *et al.*, 2001; DICKE & VAN LOON, 2000), thereby benefiting the plant. Moreover, hyperparasitoides or carnivores of the fourth trophic

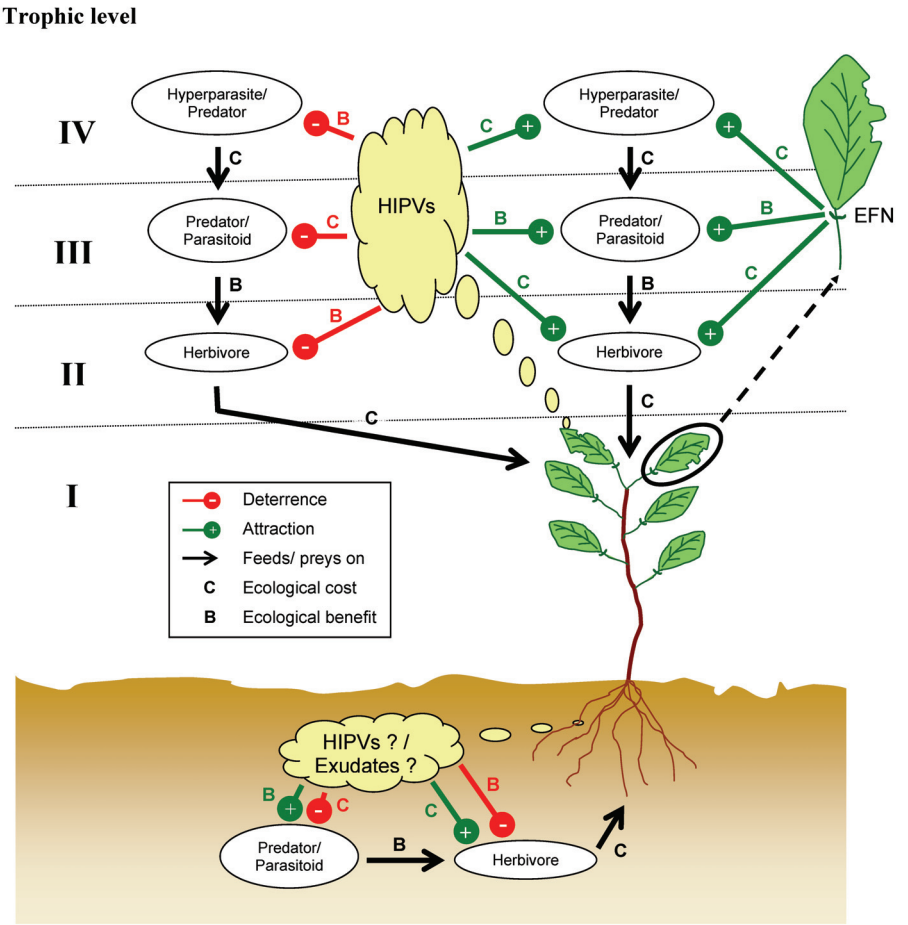


Figure 5. Potential interactions of plants with higher trophic levels via herbivore-induced plant volatiles (HIPVs) and extrafloral nectar (EFN). The stipules at the petioles of the leaves represent extrafloral nectaries. Effects mediated by HIPVs or EFN can be attraction (+) or deterrence (-) that consequently affect trophic interactions such as herbivory or predation (Arrows). Additionally, the ecological fitness costs (C) and benefits (B) of each interaction from the plant's perspective are indicated.

level could use plant-derived volatiles for the search on larger spatial scales to find their prey or host by locating potential host plants (VÖLKL & SULLIVAN, 2000). Such attraction would again impose the respective plant with ecological costs (Fig. 5).

Several plant species have the potential to defend themselves indirectly using HIPV emission and EFN secretion; both indirect defences are inducible via the octadecanoid pathway (Table 1). Comparing the widespread distribution of EF nectaries across the plant kingdom (KOPTUR, 1992) with the small number of nearly exclusively crop species for which HIPVs have been studied (DICKE, 1999c; VAN POECKE & DICKE, 2004), an increasing number of plant species that features both indirect defences can be expected. Beyond that, a wide taxonomical distribution of these plant traits may indicate the ecological success of these two induced, indirect defences.

In the ecological arms race between plants and herbivores, the latter are known for rapidly adapting to novel host plants or toxins (AGRAWAL, 2000; FRY, 1989; KARBAN, 1989) by evolving specific mechanisms to circumvent direct defences with physiological tolerance (DUFFEY, 1980; NISHIDA, 2002) or behavioural responses (DUSSOURD & DENNO, 1991; DUSSOURD & EISNER, 1987). In view of the diverse spectrum of carnivorous species which is attracted to plants expressing indirect defences, it is hard to imagine any mechanism or strategy that herbivores might evolve to cope with these highly efficient plant defenders.

5. Conclusions

We described what is currently known about molecular mechanisms of cell signalling and signal travelling, as well as the ecology and evolution of herbivore-induced plant responses. Despite the effort that has been brought to bear on this topic, the exact mechanisms as well as the ecological and evolutionary relevance still remain elusive. One reason is the diversity that exists on the genetic, biochemical, physiological, and phenotypical levels and that differs with the evolutionary background of the studied plant species. In ecological studies, even the geographical location with its particular biotic and abiotic conditions influences the outcome of a field experiment. We therefore suggest adopting model systems beyond the most frequently used ones, namely tobacco, *Arabidopsis*, maize, and Lima bean to distinguish in a more comparative way particular phenomena from general mechanisms. Such a biodiversity-oriented approach should include more non-crop species since in this case a stronger co-evolution with natural herbivores is to be expected.

Connectivity exists between multiple defences, as they are often regulated via the same signalling cascade (BALDWIN, 2001; BALDWIN & PRESTON, 1999),

leading to considerable methodological problems when individual defensive plant traits are studied. For example, the clear distinction between pure pathogen and herbivore defence pathways was undermined by the finding that the type of damage inflicted by an herbivore (chewing caterpillars versus sucking aphids) determines which defence pathway (SA versus JA) is activated. Further identification of specific elicitors and the incorporation of response mutants and transgenic plants will contribute to separate the different defence responses. These new approaches have to be complemented by creative field experiments and comparative approaches to gain better insights into emerging areas like e.g. the macroecology or community ecology of induced, indirect defences. Finally, the interactions of plants with microorganisms inside the herbivores' gut or with micro- or macrobiota belowground are promising fields of future research.

Manuscript II

Increased availability of extrafloral nectar reduces herbivory in Lima bean plants (*Phaseolus lunatus*, Fabaceae)

Christian Kost* and Martin Heil

Basic and Applied Ecology (2005) **6**, 237-248

Accepted: 5 November 2004

Department of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Beutenberg Campus, Hans-Knöll-Str. 8
D-07745 Jena, Germany

*** Corresponding author:**
Christian Kost
Dept. of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Beutenberg Campus, Hans-Knöll-Str. 8
D-07745 Jena, Germany
Phone: + + 49 - 3641 - 57 18 20
Fax: + + 49 - 3641 - 57 12 02
E-mail: ckost@ice.mpg.de

Abstract

Lima bean (*Phaseolus lunatus*) features two inducible indirect defences to protect itself against herbivores. Besides the emission of plant volatiles, extrafloral nectar is secreted to attract carnivorous arthropods to herbivore-damaged plants. The activation of both putative defences efficiently protects Lima beans from leaf damage. In a field experiment in Mexico, we studied whether extrafloral nectar alone can benefit the Lima bean under natural conditions. An artificial blend mimicking natural nectar both qualitatively and quantitatively was repeatedly applied to Lima bean tendrils. Ants, wasps and flies were significantly more abundant on treated tendrils than on untreated controls already after one week (i.e. after two treatment applications). Sticky traps were used to assess the functional groups of flying insects attracted to the Lima beans. After 24 h, 71 % of all trapped flies and 98 % of all wasps belonged to families comprising either parasitoid or predatory species. This observation suggests that also some of the flying visitors have played a role as putative defenders of Lima beans. Most of the trapped flies belonged to the families Dolichopodidae and Phoridae (each ca. one third of all individuals). Two thirds of the wasps belonged to Chalcidoidea (68 %). All ant species that had been collected manually belonged to generalist genera with *Camponotus novogranadensis* and *Cephalotes minutus* being most regularly encountered on study tendrils. An additional experiment, where both 'nectar' and 'control' tendrils were treated with artificial nectar, revealed that ants responded with an increased abundance on tendrils that had experienced the 'nectar' treatment before. After 25 days, the treated tendrils showed a significantly reduced herbivory as compared to controls. The mere presence of increased amounts of extrafloral nectar thus can benefit the Lima bean under natural conditions.

Zusammenfassung

Die Limabohne (*Phaseolus lunatus*) verfügt über zwei induzierbare, indirekte Verteidigungsformen zur Abwehr von Herbivoren. Neben der Emission volatiler Verbindungen ist die Limabohne zusätzlich dazu in der Lage, extrafloralen Nektar zu sezernieren. Beides dient der Anlockung von Fraßfeinden zu den von Herbivoren befallenen Pflanzen. In einem Freilandexperiment in Mexiko wurde untersucht, ob die Limabohne unter natürlichen Bedingungen von der Sekretion extrafloralen Nektars profitiert. Hierzu wurde ein künstliches Nektargemisch wiederholt auf Limabohnenranken aufgetragen, welches natürlichen Nektar quantita-

tiv und qualitativ imitierte. Bereits nach einer Woche (d.h. nach zwei Behandlungen) war die Abundanz von Ameisen, Fliegen und Wespen auf behandelten Ranken signifikant höher als auf unbehandelten Kontrollranken. Zur Erfassung der zur Limabohne angelockten fliegender Insekten sowie deren Zugehörigkeit zu funktionellen Gruppen wurden die Versuchsranken mit Klebefallen bestückt. Mehr als zwei Drittel der nach 24 h gefangenen Fliegen und 98 % aller Wespen gehörten parasitoidisch oder räuberisch lebenden Fliegen- bzw. Wespen-Familien an. Diese Beobachtung legt nahe, dass nicht nur Ameisen, sondern auch einige der gefangenen fliegenden Besucher eine Rolle als potentielle Verteidiger der Limabohne gespielt haben könnten. Von den gefangen Fliegen gehörten die meisten den Familien Dolichopodidae und Phoridae (je ca. ein Drittel aller gefangenen Individuen) an, wogegen die Chalcidoidea zwei Drittel (68 %) der gefangenen Wespen ausmachten. Unter den durch Handaufsammlung gefangenen Ameisen gehörten *Camponotus novogranadensis* und *Cephalotes minutus* zu den am häufigsten auf behandelten Ranken angetroffen Arten. Ein zusätzliches Experiment, in dem das künstliche Nektargemisch sowohl auf 'Nektar'- als auch auf 'Kontroll'-Ranken aufgebracht wurde, ergab, dass die Ameisen mit einer erhöhten Abundanz auf solchen Ranken reagierten, die bereits vorher die ‚Nektar'-Behandlung erfahren hatten. Nach 25 Tagen zeigten behandelte Ranken signifikant weniger Blattfraß im Vergleich zu unbehandelten Kontrollranken. Die bloße Erhöhung der Menge an extrafloralem Nektar reichte offensichtlich dazu aus, unter natürlichen Bedingungen wachsenden Limabohnen einen Vorteil zu verschaffen.

Key words: ants, anti-herbivore defence, artificial nectar, induced defence, Leguminosae, plant-herbivore interaction, sugar, Mexico

Introduction

Plants have developed a large number of different strategies to defend themselves against herbivores. Such strategies can be divided into direct or indirect defences. Direct defences per definition directly exert negative impacts on herbivores. They comprise, e.g., spines, thorns, trichomes, waxes and a large diversity of secondary plant metabolites. On the other hand, indirect defences are plant characteristics that include higher trophic levels such as parasitoids or predators of the herbivores, which then fulfil the defensive function (PRICE *et al.*, 1980).

Extrafloral nectaries are nectar-secreting organs that are not directly involved in pollination, yet play a vital role in maintaining other mutually beneficial relationships among plants and animals (ELIAS, 1983). In general, extrafloral nectar (EFN) is believed to function as an indirect defensive mechanism. It is mainly composed of mono- and disaccharides and amino acids (GALETTO & BERNARDELLO, 1992; HEIL *et al.*, 2000b; RUFFNER & CLARK, 1986). Besides ants, which constitute the main portion, other insects such as ichneumonid and braconid wasps (BUGG *et al.*, 1989; GENTRY, 2003; STAPEL *et al.*, 1997) or mosquitoes (FOSTER, 1995) utilize this food source as well, resulting in varying degrees of anti-herbivore protection. The presence of ants, for example, has been repeatedly shown to enhance the protection of entire plants or plant parts (BENTLEY, 1977a; HEIL *et al.*, 2001; SOBRINHO *et al.*, 2002), whereas other visitors may act as commensals or even parasites (HEIL *et al.*, 2004a; O'DOWD, 1979).

Studies investigating the role of extrafloral nectar as an indirect defence ace considerable methodological problems. In the majority of studies this issue was addressed by excluding ants using sticky barriers such as Tangletrap® (CUAUTLE & RICO-GRAY, 2003; LABEYRIE *et al.*, 2001; STEPHENSON, 1982). The drawback of this attempt is that crawling herbivores are excluded as well (FREITAS *et al.*, 2000; MACKAY & WHALEN, 1998) and that this systematic error finally can lead to an underestimation of the 'true' effect. The second way to approach this issue is using plant-derived elicitors such as jasmonic acid that induce extrafloral nectar flow (HEIL *et al.*, 2001) or to induce EFN secretion by natural herbivory (NESS, 2003). Such induction phenomena often act via the octadecanoid pathway, which is well known to not only regulate the flow of extrafloral nectar, but also to mediate many other induced direct or indirect defences, such as, e.g., the change of chemical constituents in attacked plant tissues, or the production of volatile organic compounds (VOCs) to attract natural enemies of the herbivores (KARBAN & BALDWIN, 1997). Consequently, the studies conducted so far suffered from differ-

ent methodological problems: Exclusion experiments run the risk of accidentally barring crawling herbivores and thus underestimating the defensive effect, those inducing EFN secretion may overestimate it due to undesigned inductions of additional direct or indirect defences.

A possibility that circumvents all the abovementioned difficulties is the artificial increase of extrafloral nectar by exogenous application of sugar solution. Amongst others (BENTLEY, 1976; TEMPEL, 1983), this approach was followed up by Jacob & Evans (1998), who sprayed alfalfa plants (*Medicago sativa*) with an aqueous solution of sucrose. Two days later, significantly higher numbers of adult parasitoids as well as an increase in the parasitation rate of herbivorous weevils were observed in plots sprayed with sugar solution as compared to control plots, which had been sprayed with water only.

Phaseolus lunatus L. (Lima bean, Fabaceae) is a common model plant used in genetic, biochemical or ecological studies. Besides the emission of VOCs that follows herbivore attack and attracts carnivorous animals (DICKE, 1994; DICKE *et al.*, 1990b; DICKE *et al.*, 1993), the Lima bean features extrafloral nectaries (HEIL, 2004a). Although several aspects of VOC emission of Lima beans have been studied intensively in laboratory or greenhouse experiments, almost nothing is known on the ecological function of its extrafloral nectaries and of VOC-emission in nature. A previous study demonstrated that regularly treating wild Lima bean with jasmonic acid and thereby inducing both the production of extrafloral nectar and VOCs benefits the plants at their natural growing site (HEIL, 2004a). However, it remained unclear which insects were involved in this interaction on both the side of herbivores and defenders. Moreover, it was not clear whether extrafloral nectar alone could also exert a defensive effect.

The aim of the present study was to follow the approach of JACOB and EVANS (1998), yet applying artificial nectar directly onto or nearby extrafloral nectaries in an amount and quality that mimicked the natural occurrence of extrafloral nectar. The following questions were addressed: i) What kinds of insects are attracted to the artificial nectar applied to Lima beans? ii) Does extrafloral nectar alone exhibit a beneficial effect for the Lima bean at its natural growing site?, and iii) Does the presence of ants on Lima bean tendrils depend on their previous foraging experience (i.e. do they appear in increased numbers where more nectar can be expected)?

Material and Methods

Study site and species

The study was conducted in the coastal area of the state of Oaxaca, Mexico, a location close to the centre of the genetic diversity of wild Lima beans (SALGADO *et*

al., 1995). Two sites, some 15 km northwest of Puerto Escondido, were selected. These two sites were about 3 km apart from each other and 1 to 2 km away from the Pacific Ocean. Both study sites represented waysides along dirt roads leading to extensively used pastures or plantations, with site 1 featuring denser vegetation and site 2 being much more exposed to direct sunlight. The two sites are identical with those of a previous study (HEIL, 2004a). The climate in the study area is characterized by one main rainy season from June to October, which follows a bimodal distribution peaking in July and September. Annual rainfall averages between 1,000 and 1,400 mm and the mean annual temperature is 28 °C (STRÄßNER, 1999).

Wild Lima bean (*Phaseolus lunatus* L., Fabacecae) grows as a twining vine or herbaceous bush, entwining trees and shrubs along open sites such as forest edges or along roadsides. The Lima bean grows in part as a perennial plant, although the aboveground parts usually die during the dry season. In June or July the plants start to germinate or bud, and the first inflorescences usually appear in October or November. Depending on water supply, the production of flowers and fruits ends between February and April (HEIL, 2004a). As many other Fabaceae, the Lima bean bears extrafloral nectaries. These are located on its bracts, or arranged pairwise at the stipules of the trifoliate leaves as well as at the petioles of the individual leaflets (HEIL, 2004a). Voucher specimens are deposited at Herbario MEXU (UNAM, Mexico City, Mexico) and in the personal collection of M. HEIL.

Experimental design

In the beginning, 23 pairs of Lima bean tendrils were selected. Eleven of these pairs were located at site 1 and twelve at site 2. Due to the tangled growth of Lima bean it was not possible to distinguish single plant individuals. Therefore, paired tendrils either belonged to one single plant individual or to two adjacent plants with a maximum distance of 2 m. Tendrils of each pair were stretched along two strings for further growth. One tendril per pair was randomly assigned to treatment ‘nectar’, the other one served as an untreated control. ‘Nectar treatment’ means an aqueous solution of artificial nectar was applied directly on the extrafloral nectaries using an Eppendorf pipette. ‘Nectar’ tendrils were treated every 3 to 4 d from 27.10.2003 until 18.11.2003, resulting in six application events. During this time, the initial number of pairs was reduced to a final sample size of 17 due to human impact.

Artificial nectar

To mimic the extrafloral nectar production of the Lima beans qualitatively and quantitatively, both the secretion rate and the chemical composition of extrafloral nectar

had to be assessed. Lima bean plants growing at the aforementioned study sites had been induced by spraying with 1 mmol aqueous JA-solution (HEIL, 2004a). After 24 h, the nectar production rate of these plants was quantified as amounts of secreted soluble solids, by quantifying the nectar volume with micro capillaries and the nectar concentration with a refractometer (HEIL *et al.*, 2000b; 2001). The nectar secretion rate was then related to the secreting leaves and their respective dry weight. The estimated secretion rate for a strongly induced Lima bean leaf averaged about 1.5 mg soluble solids g^{-1} leaf dry mass 24 h^{-1} as compared to 0.02 mg g^{-1} 24 h^{-1} for an uninduced control leaf (HEIL, 2004a).

The subsequent analysis of EFN focused on sugars and amino acids, as they comprise the most important components of extrafloral nectar (BAKER *et al.*, 1978; GALETTO & BERNARDELLO, 1992; RUFFNER & CLARK, 1986). Other potential nectar constituents such as proteins, vitamins or lipids were not considered. To analyse sugars and amino acids as their trimethylsilylated derivatives, 50 µl *N*-Methyl-*N*-(trimethylsilyl)-rifluoracetamide (MSTFA) was added to a 2 µl nectar sample and heated for 1 h to 60 °C. The samples were diluted with dichloromethane in a rate of 1:100. Sugars from commercial sources (Sigma) served as standards: 2 µl of an aqueous solution (100 mg ml^{-1}) was treated as described above.

Samples were analysed on a GC-Trace mass spectrometer (Thermo Finnigan) using a DB 5 column (15 m x 0.25 mm x 0.25 µm; Alltech, Unterhaching, Germany). Sugars were separated with the following program: 120 °C for 2 min isothermal, then at 7 °C min^{-1} to 250 °C, temperature of the injection port of 220 °C and temperature of the GC-interface at 280 °C. Simultaneous analysis of sugars and amino acids required a modified program: 40 °C for 2 min isothermal, then at 10 °C min^{-1} to 120 °C, after that at 7 °C min^{-1} to 250 °C and a GC operating with a split-ratio of 1:10. This analysis identified glucose, fructose and sucrose as the main components of the extrafloral nectar blend, with amino acids being below the detection limit (i.e. < 1.3 pmol/µl). The blend of artificial nectar, which was adjusted to the cumulated secretion rate of one leaf for three days, consisted of 4.01 g sucrose l^{-1}, and 24.24 g l^{-1} of each glucose and fructose. Every three to four days, 40 µl of this blend were applied directly to the extrafloral nectaries of every leaf of the 'nectar' tendrils.

Insect counts

The first census of the arthropods on the 23 selected pairs of tendrils was performed immediately before the first treatment (27.10.03 for site 2 and 29.10.03 for site 1). Both censuses were performed at the same time of day. Numbers of ants, wasps or flies present on the plants were

recorded, as they represented the most abundant groups.

Two additional censuses were performed in the course of the experiment, to test the influence of the 'nectar' treatment on the insect community. The first census started d 7 of the experiment (7.11.03) at site 1. Ten pairs of tendrils were selected, which had experienced two treatments until then. The first census was performed prior to the nectar application at 8:00 am. Thereafter all 'nectar' tendrils were treated and insects on all pairs were counted repeatedly every 2 or 3 h until midnight. Two additional censuses were performed at 9:00 and 10:00 am on the following two days, resulting in a total of 14 monitorings. The same was done with 10 pairs of plants at site 2. This second series started at d 18 after the onset of the experiment (14.11.03) and consisted of a total of 13 censuses.

Defensive effect

The herbivory rate in percent leaf loss was chosen as a fitness-relevant parameter. This parameter was quantified by assigning leaves to one of the following ranges of missing leaf area: 0 %, 1 - 5 %, 6 - 10 %, 11 - 25 %, 26 - 50 % and 51 - 100 %.

At the beginning of the experiment (24.10.03), the herbivory rate for all leaves present on the studied tendrils was assessed as a reference. After 25 days, the remaining tendrils were checked again: The herbivory rate of all leaves was estimated when the total leaf number per tendril was < 25, otherwise 25 leaves were randomly selected and the herbivory rate assessed.

Finally, average values were calculated for the two estimations and the difference between these two was used to measure the development of leaf damage. To test the accuracy of this method, the missing leaf area of 74 randomly chosen leaves was both estimated as mentioned above and quantified as follows: First, leaves were dried between sheets of paper for transport (leaves shrink less than 5 % during this procedure). The leaves then were scanned together with a reference area of 1 cm^2 (hp scanjet 8200 scanner). The resulting digital pictures were analyzed with the graphics software package Corel photo paint version 8.232. By outlining the area of both a given trifoliate leaf and the reference area, the corresponding leaf area could be determined, as indicated by the number of pixels. The missing leaf area was outlined accordingly. In cases of complete leaflets missing, the corresponding area was estimated since both the areas of the left and the central leaflet ($r = 0.93$, $p < 0.001$, $n = 58$, $y = 0.8397 x + 20786$) as well as the two lateral leaflets showed a strong correlation ($r = 0.95$, $p < 0.001$, $n = 56$, $y = 0.8361 x + 13361$). Finally, the percentage of missing leaf area was computed from missing and total area. The close linear relationship between the measured and estimated

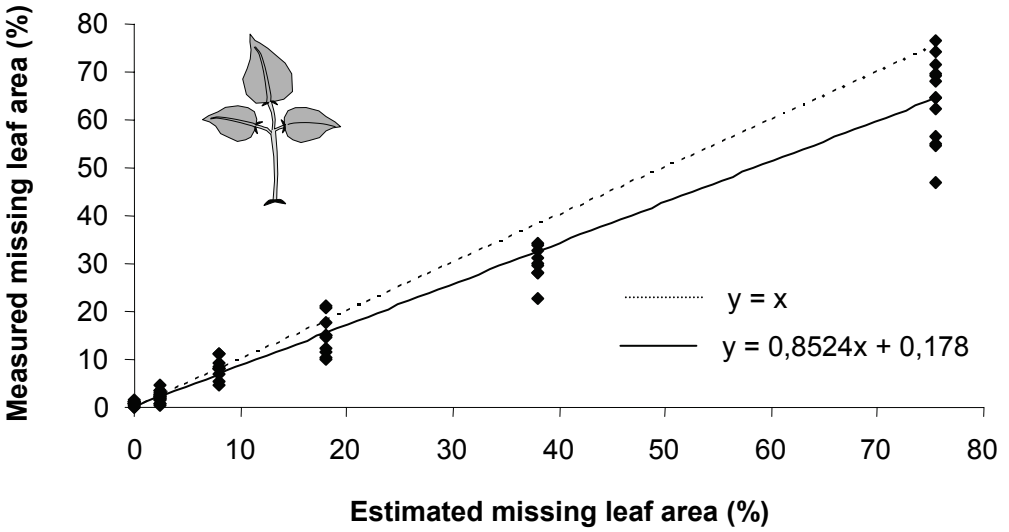


Figure 1. Comparison between estimated and measured leaf area missing of 74 leaves of *Phaseolus lunatus*. Leaf area was estimated by assigning leaves to the closest of six different classes of missing leaf area (0 %, 1 - 5 %, 6 - 10 %, 11 - 25 %, 26 - 50 % and 51 - 100 %), of which the corresponding mid-points are displayed. For exact measurements digitized pictures of leaves were analysed by relating pixel numbers of outlined leaves to a reference area. A regression was calculated (solid line) to relate the estimated to the measured leaf area ($r = 0.981$, $p < 0.001$, $n = 74$). The axial bisector (dashed line) shows the theoretically perfect correlation.

missing leaf areas (Fig. 1) allowed the simplified determination of the missing leaf area by estimation.

Insect collection

Ants, wasps and flies were the insect groups most regularly encountered on experimental Lima bean tendrils. To study their affiliation to functional groups, specimens of all three groups were collected. Ants were sampled at each of the two study sites from randomly chosen Lima bean clusters that were directly neighbouring the study tendrils. All ants that showed up within approximately 45 min were collected manually with tweezers. Flies and wasps were collected with sticky traps that consisted of 100 cm^2 pieces of green plastic foil that had been coated with a thin layer of a trapping adhesive (Tangletrap®). Two such sticky traps were attached with plastic strings to each of 14 'nectar' and 'control' tendrils that were equally distributed between the two study sites. After 24 h of exposure, the traps were re-collected and the insects transferred to 75 % ethanol. Ants were determined to species level whenever possible by MANFRED VERHAAGH, Staatl. Museum für Naturkunde Karlsruhe. Flies and wasps were identified to family level using keys and information provided by ARNETT (2000) and SCHAEFER *et al.* (1994) for insect families and NOYES (2003) for chalcid families. On the

basis of the natural history information provided by HONOMICHL (1996) for flies and wasps, RETTENMEYER (1961) and DISNEY (1994) for phorid flies as well as RICO-GRAY (1993) and BROWN (2000) for ants, the collected insects were assigned to the following guilds according to nutritional or functional aspects: Detrivore (D), Frugivore (F), Herbivore (H), Parasitoid (P), Predator (R) or utilization of other plant-derived resources including floral or extrafloral nectar, pollen and honeydew (S).

Nectar experiment

To assess whether increased ant numbers on the nectar-treated tendrils resulted from the actual amount of artificial nectar alone, or whether the frequency of ant foragers had been adjusted to their previous foraging experience, nine pairs of tendrils (three at site 1 and six at site 2) were selected and both the 'nectar' and the 'control' tendril treated with artificial nectar. The abundance of ants, wasps and flies was assessed in advance, and every 30 to 45 minutes after the treatment, resulting in 9 to 11 countings per tendril pair.

Statistical analysis

The approach of using adjacent pairs of 'nectar' and 'control' tendrils allowed the use of the Wilcoxon test for matched pairs whenever single or averaged values between the two treatments had to be compared. The influence of the factor 'site' was verified by applying Mann-Whitney U-tests to the census data of different insect groups which were assessed before the onset of the experiment (i.e. 27.10.03 for site 2 and 29.10.03 for site 1). Data were pooled for tendrils that afterwards were assigned to the two treatments since no treatment had been administered at that time. Tests for the factor 'time' were done by comparing insect numbers assessed before the start of the experiment, with one comparable census (i.e. same time of day) seven days (site 1) or 18 days (site 2) after the start of the experiment. All statistical evaluations were done using SPSS 10.0.

Results

Effects of the 'nectar' treatment on insect abundance

The application of artificial nectar resulted in a significant increase in the abundance of ants, wasps and flies on nectar tendrils compared to control tendrils already after seven days (i.e. after two treatments, Fig. 2). The second assessment of insect abundance starting from day 18 revealed an even more pronounced increase in the number of ants. However, no statistically significant difference between 'nectar' and 'control' tendrils could be detected for wasps and flies during the second census. The three focal insect groups showed a strong diurnal activity pattern with a subsequent decline during the night for wasps

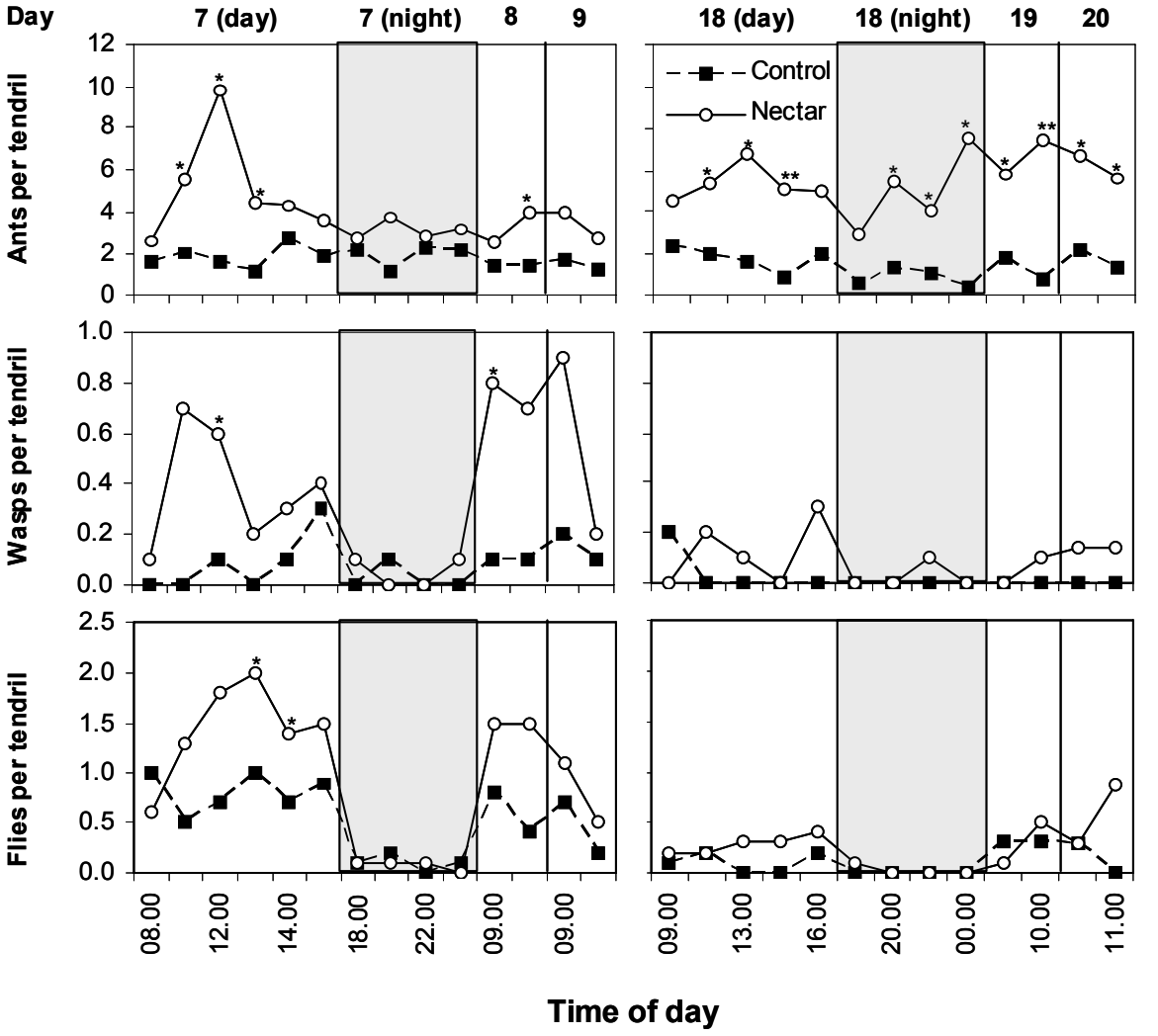


Figure 2. Temporal pattern of the three most abundant insect groups on tendrils treated with artificial nectar and untreated controls, recorded at Site 1 (left) and Site 2 (right). The first census at each site shows insect numbers before the application of artificial nectar; the following censuses represent insect abundance thereafter. Insect numbers for 10 pairs of plants at a time were averaged for each census. Censuses were performed at three consecutive days starting at Site 1 on day seven after the beginning of the experiment and on Site 2 on day 18. Significant differences between corresponding pairs of tendrils are indicated as * $p < 0.05$, or ** = < 0.01; Wilcoxon pair test.

and flies, but no strong relation to the time of day for ant activity (Fig. 2).

Insect censuses represented not only two different dates (i.e. 7-9.11.03 and 14-16.11.03), but they were also performed at different sites. Therefore, a priori differences of insect abundance (factor 'site') and the changes of insect abundance in the course of the experiment (factor 'time') were assessed. Differences between sites were determined for the abundances of ants, wasps and flies present on study tendrils at the onset of the experiment. As no treatment had been administered at

that time, insect abundances on the tendrils which were later subjected to the treatments 'nectar' and 'control' were pooled. The corresponding analysis included 23 tendrils (n = 11 at site 1 versus n = 12 at site 2) and revealed that abundances of wasps and flies were significantly higher at site 1 as compared to site 2 (Mann-Whitney-U-tests in both cases $P < 0.001$), whereas no such difference between sites could be detected for ants (Mann-Whitney-U-test $P = 0.515$).

The putative influence of the factor 'time' was determined by comparing insect abundances on 'control' tendrils before the onset of the experiment with those found seven (site 1) or 18 days (site 2) later on the same untreated tendrils. No statistically significant influence of the factor 'time' could be detected for the abundance of any of the focal insect groups ($P > 0.05$ according to Wilcoxon pair tests with n = 10 for both sites). Hence the abundance of ants was neither influenced by the factors 'site' nor 'time', whereas the abundance of wasps and flies was heavily influenced by *a priori* 'site'-effects.

As ants responded strongest to the application of artificial extrafloral nectar, an additional experiment was conducted to clarify whether their increased presence on nectar tendrils depended on their previous foraging experience? In this case, the presence of ants would be higher on tendrils on which increased amounts of nectar had been available before (i.e. 'nectar' tendrils). Artificial nectar was applied to both the 'nectar' tendrils, which so far had been treated six times every 2 to 3 days, and to the 'control' tendrils. The subsequent monitoring of ant activity on both classes of tendrils revealed a very similar pattern of ant response: Ant activity peaked 1.3 h after nectar application, thereafter declined (Fig. 3). However, the intensity of ant response was significantly lower on 'control' compared to 'nectar' tendrils.

Visitors of the Lima bean

The sticky trap experiment confirmed the observation that flies and wasps played an important role as visitors of the Lima bean: 44 % of all insects trapped were dipterans and 26 % hymenopterans (Table 1). Other groups trapped were mainly Coleoptera (11 %), Auchenorrhyncha (9 %) or Araneida (6 %). Although there was no significant difference detectable between control and nectar tendrils for any of the trapped arthropod groups (Wilcoxon signed rank test $P > 0.05$), a more detailed analysis of the functional groups indicated a large proportion of putative beneficial insects visiting the Lima bean. Among the flies, Dolichopodidae (31 %) and Phoridae (27 %) were the most abundant families (Table 1). The Dolichopodidae are known to prey on smaller insects or insect larvae that live in the vegetation along coastal areas. The family Phoridae covers many predatory or parasitoid species of mainly

Table 1. Wasps and flies trapped on experimental clusters with sticky traps and ants collected manually from Lima bean clusters adjacent to the experimental clusters.

Order	Taxon	Number (ind.)	Guild					
			R	**P**	**S**	**F**	**H**	**D**
Diptera								
	Dolichopodidae	47	l/a		a			
	Phoridae	40	l	a	a			
	Tachinidae	11		l	a			
	Chloropidae	9	l	l	a		l	
	Culicidae	6			a			l
	Drosophilidae	4				l/a		
	Platystomatidae	3			a			l
	Agromyzidae	2			a		l/a	
	Canacidae	2	?					
	Lauxaniidae	2			a			a
	Muscidae	2	l		a			l
	Psychodidae	2			a			l
	Scenopinidae	2	l		a			l
	Syrphidae	2	l		a			l
	Others	14						
Hymenoptera								
Wasps	Chalcidoidea	60	l/a	a				
	Braconidae	8	l/a	a	a			
	Ichneumonidae	3	l/a	a	a			
	Dryinidae	2	l/a	a				
	Sphecidae	2	l/a					
	Others	5						
Ants	*Camponotus novogranadensis*	n. d.	l		a			
	Camponotus (Myrmobrachys) sp.	n. d.	l		a			
	Cephalotes minutus	n. d.	l		a			
	Crematogaster sp.	n. d.	l		a	a		
	Monomorium sp.	n. d.	l		a	a		
	Paratrechina longicornis	n. d.	l		a			
	Pseudomyrmex sp. 1	n. d.	l		a			
	Pseudomyrmex sp. 2	n. d.	l		a			

Numbers of wasps and flies refer to the total number of insects trapped and was not determined for ants (**n. d.**). The observed flies, wasps and ants were assigned to the following guilds according to nutritional or functional aspects: **D**, detrivore; **F**, frugivore; **H**, herbivore; **P**, parasitoid; **R**, predator/entomophaga; or **S,** utilization of other plant-derived resources including floral or extrafloral nectar, pollen and honeydew. The affiliation to a guild is further differentiated by the developmental stage of each taxon (**l** = larval, **a** = adult) that features the respective trait.

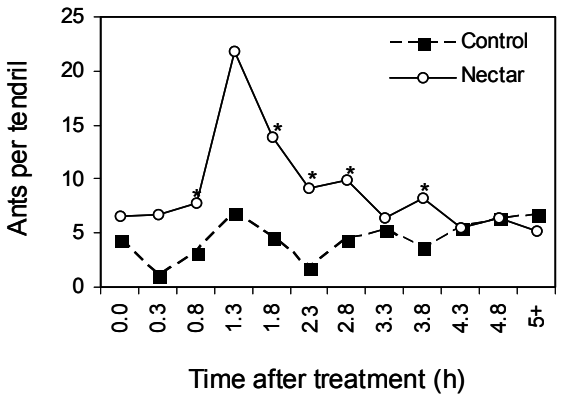


Figure 3. Temporal pattern of the presence of ants on nine pairs of tendrils (three from Site 1 and six from Site 2) after treating both 'control' and 'nectar' tendrils with artificial nectar. Ant numbers were averaged for each census. Significant differences between corresponding pairs of tendrils are indicated as * $p < 0.05$; Wilcoxon pair test.

predatory and parasitoid life habits. Among the Hymenoptera this trend became even more conspicuous: Except one single individual of the Cephidae (Symphyta), all trapped Hymenoptera (98 %) showed

either parasitoid or predatory life habits. Here, members of the superfamily Chalcidoidea were the most abundant group, which alone already contributed 68 % to all hymenopterans trapped. These parasitoid wasps belonged to 14 different families with the Pteromalidae being the most abundant (27 %).

Among ants, *Camponotus novogranadensis*, *Cephalotes minutus* and *Crematogaster* sp. were most regularly encountered on study tendrils (C. KOST, personal observation). The remaining species could only be observed occasionally and thus seem to be of minor importance. All ants collected were generalists, which apart from their preference for sugar sources such as extrafloral nectar, are well known to prey on various arthropods (Table 1).

Effect of the 'nectar' treatment on the herbivory rate

The application of artificial nectar resulted in a significant reduction of the herbivory rate in treated versus control tendrils (Fig. 4, P = 0.01; Wilcoxon pair test for pooled pairs). However, this protective effect differed between the two sites (Fig. 4): It was very obvious at site 1 (Wilcoxon pair test: P = 0.047), whereas the

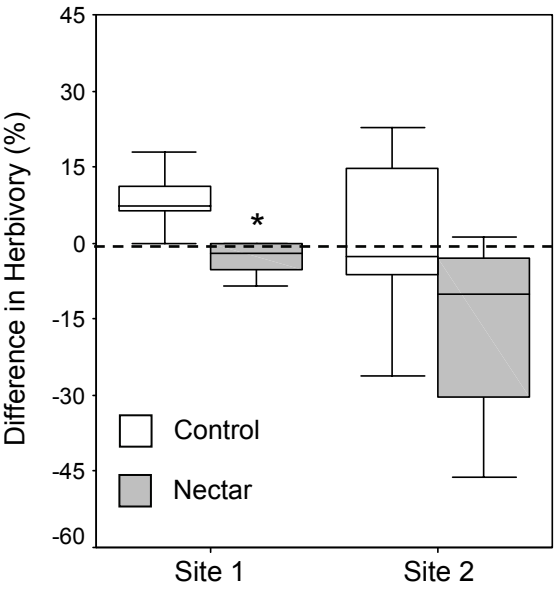


Figure 4. Effect of the application of artificial nectar on herbivory of *Phaseolus lunatus* at the two study sites. Herbivory is presented as relative differences (day 0/day 34) in the estimated herbivory rate (%) for 17 pairs of Lima bean tendrils (n = 10 pairs for Site 1 and n = 7 pairs for Site 2, * P < 0.05; Wilcoxon pair test).

same trend but no significant difference could be detected for site 2 (Wilcoxon pair test: P = 0.091).

Discussion

The main purpose of the present study was to unravel whether the secretion of extrafloral nectar benefits the Lima bean at its natural growing site. Many studies on this topic have already been conducted before, but most were hampered by methodological problems such as i) unintended exclusion of crawling herbivores, ii) not taking into account putative influences of flying plant defenders, and iii) simultaneous induction of other defensive mechanisms. In order to focus on defensive effects of EFN only, artificial nectar that mimicked the nectar secretion of induced Lima bean plants in terms of amount and chemical composition was applied to Lima bean plants.

The application of artificial nectar resulted in a significantly increased abundance of ants, wasps and flies on treated compared to control tendrils after only seven days (Fig. 2). Most of the wasp and fly families encountered belonged to families that exclusively or at least partially comprise parasitoid or predatory species (Table 1). On this account, the observed defensive effect may not only be due to the increased presence of ants, but could also be exerted by flying defenders that are attracted to the extrafloral nectaries. The following findings may also point into this direction: A comparison of insect abundances between the two sites before the start of the experiment revealed significantly higher frequencies of wasps and flies at site 1 than at site 2, whereas no such difference between sites could be detected for ants. At the end of the experiment, site 1 showed a strongly reduced herbivory level, while at site 2 only a trend towards a reduced herbivory level of treated tendrils could be observed (Fig. 4). The protective effect was thus strong at the site where, besides ants, also wasps and flies showed increased activities, but it was weak at the site where mainly ants were attendant to fulfil a defensive function. Obviously, flying defenders added significantly to the overall defensive effect. Which of these two groups (flies and wasps) contributed most to the observed defensive effect?

It seems likely that the attracted wasps contributed more to this effect than the attracted flies. All flies belonging to the family of Dolichopodidae were relatively small and thus may not have been able to prey on larger herbivores. Furthermore, the Phoridae are known not only to parasitize herbivorous species (RETTENMEYER, 1961) but also ants (DISNEY, 1994). An increased abundance of phorid flies might therefore also have led to a reduction of the ‘true’ defensive effect.

Most of the studies regarding the defensive effect of EFN focused on ants,

without taking into account influences of other putative plant defenders such as spiders (RUHREN & HANDEL, 1999) or parasitoids (PEMBERTON & LEE, 1996). Besides this study, only one study is available to date where the defensive roles of several groups of plant defenders have been studied simultaneously: CUAUTLE & RICO-GRAY (2003) showed that either ants or wasps exerted beneficial effects on the extrafloral nectary bearing plant *Turnera ulmifolia* when selectively excluded; when both insect groups had access, however, their effects were not additive.

Extrafloral nectaries secrete small amounts of nectar throughout the day. Nectar can be secreted in diurnal patterns that are characterised by short peaks (HEIL *et al.*, 2000b; RAINE *et al.*, 2002) or at relatively constant rates throughout day and night (BENTLEY, 1976, 1977b). The exact mode of nectar secretion is not known for *Phaseolus lunatus*. An application of artificial nectar at intervals of 3 to 4 d, however, does most likely not match the natural situation. Yet, the attracted insects may have responded to the increased sugar amounts itself rather than being visually attracted to the relatively large droplets of sugar solution, since already 10 to 15 minutes after nectar application the solvent water was completely evaporated leaving behind only the sugars.

Even though the applied nectar was consumed very rapidly (C. KOST, personal observation), the abundance of ants, wasps and flies was sustainably increased during the time of application: Even at the third day following nectar application, all three insect groups still showed increased abundances (Fig. 2).

As ants were most dominant, an experiment was performed to verify, whether this sustainable increase in ant numbers on ‘nectar’ tendrils was due to their ability to respond with a modification of their foraging behaviour based on previous experiences. The ‘nectar experiment’, in which artificial nectar was applied to both ‘nectar’ and ‘control’ tendrils at the end of the experiment, revealed a similar pattern of ant response for both types of tendrils (Fig. 3). However, the intensity of the response was significantly increased for tendrils that had experienced the nectar treatment before. Obviously, the ants responded positively by preferentially foraging on tendrils that usually were characterised by an increased availability of extrafloral nectar.

This effect could be explained by increased time periods the ants spend on plants that supply EFN more regularly, with increased visitation frequencies of foraging ants, or with a more efficient recruitment to these tendrils. Experimental findings seem to support the second alternative: The time foraging ants spend at a food source depends on flow rates experi-

enced previously and decreases with increasing nectar flow rates (SCHILMAN & ROCES, 2003). Increased flow rates lead at the same time to an increase of the volume collected. The ecological function of such a foraging behaviour may be a defence-by-exploitation strategy. Resident ants avoid competition against other nectar consumers by systematic and frequent visitation, thus leading to an exploitation of the food source (DREISIG, 2000). Such an increased visitation frequency advances the probability of ant attendance, thereby facilitating the protective effect. This strategy seems to be characteristic not only for the species tested in the studies mentioned before, but for all generalistic ants (F. ROCES, personal communication).

The 'control' tendrils were characterized by a certain level of 'background' EFN secretion. The secretion rate of 18 Lima bean plants that were untreated and adjacent to the experimental tendrils at site 1 ranged about ca. 0.36 mg soluble solids g^{-1} 24 h^{-1} (C. KOST, unpublished data). Adding the constitutive secretion of EFN to the amount of applied artificial nectar results in c. 1.06 mg soluble solids g^{-1} 24 h^{-1} of nectar being available on 'nectar' tendrils. Earlier measurements (2002) at the same site revealed a secretion rate of c. 0.02 mg soluble solids g^{-1} 24 h^{-1} for untreated and ca. 1.5 mg soluble solids g^{-1} 24 h^{-1} for Lima bean plants induced with jasmonic acid (JA) (HEIL, 2004a). This comparison illustrates that the total amount of nectar, which was available on 'nectar' tendrils, did not exceed the physiological limits of nectar production in Lima beans. Moreover, since also 'control' tendrils produced certain amounts of EFN, the protective effect of EFN secretion that became obvious at the end of the experiment was systematically underestimated and thus provides a conservative measure of this defensive trait.

In the present study Lima bean plants benefited from EFN secretion in nature. Since not only ants but also flying insects were attracted to the artificial nectar, these may also have contributed to the observed protective effect. Further studies are required to elucidate which of the observed flying insects are beneficial to the Lima bean and which ones even cause ecological costs by consuming EFN without exerting a beneficial effect.

Acknowledgements

The authors thank JANINE RATTKE for analysing nectar probes and MANFRED VERHAAGH for support in ant determination. We are grateful to WILHELM BOLAND for the possibility to use all the facilities of the MPI and to PAULO S. OLIVEIRA, JANINE RATTKE, VICTOR RICO-GRAY and two anonymous referees for helpful comments on previous versions of the manuscript. The authors especially appreciate valuable logistic support in the

field received by PERLA MONICA MARTINEZ CRUZ (Universidad del Mar Puerto Escondido). Financial support by the German Research Foundation (DFG grant He3169/2-1, 2) and the Max-Planck-Society is gratefully acknowledged.

Manuscript III

Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants

C. Kost[1]* and M. Heil[1,2]

Journal of Ecology, accepted

Accepted: 6 December 2005

[1] Department of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Hans-Knöll-Str. 8
Beutenberg Campus
D-07745 Jena, Germany

[2] Present address:
Department of General Botany
University Duisburg-Essen
FB BioGeo - Botanik
Universitätsstr. 5
D-45117 Essen, Germany

*** Corresponding author:**
Christian Kost
Department of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Beutenberg Campus
Hans-Knöll-Str. 8
D-07745 Jena, Germany
Phone: + + 49 - 3641 - 57 18 20
Fax: + + 49 - 3641 - 57 12 02
E-mail: ckost@ice.mpg.de

Summary

1 Many plant species respond to herbivory with an increased emission of volatile organic compounds (VOCs), which attract carnivorous arthropods and thereby function as an indirect defence. Whether neighbouring plants positioned downwind 'eavesdrop' on such airborne cues and tailor their defences accordingly, remains controversial.

2 We used native Lima bean plants (*Phaseolus lunatus*) to investigate whether herbivore-induced VOCs induce another indirect defence strategy, i.e. the secretion of extrafloral nectar (EFN) in conspecific plant neighbours, and whether this enhances the defence status of the receiving plant under natural conditions.

3 EFN secretion was induced by VOCs released from herbivore-damaged bean tendrils as well as by a synthetic VOC mixture resembling the natural one. One constituent of the herbivore-induced blend - namely the green leaf volatile (3*Z*)-hex-3-enyl acetate - was identified as sufficient to elicit the defence reaction.

4 A long-term experiment was conducted to compare the defensive effect of EFN alone with the VOC-mediated effect (EFN induction plus attraction of plant defenders). The results indicate that Lima bean benefits from both indirect defences. Repeated treatment of tendrils with either an artificial blend of VOCs or EFN correlated with a higher cumulative number of predacious and parasitoid insects attracted (i.e. ants and wasps) as well as with less herbivore damage and increased productions of inflorescences and leaves.

5 Our results demonstrate that one indirect defence mechanism can induce another one in conspecific plants, and that Lima bean plants can benefit from this VOC-induced EFN secretion under natural conditions. Regarding the wide taxonomic distribution of both extrafloral nectaries and the capability of plants to release VOCs upon herbivory, airborne signalling may represent a common mechanism of plants to regulate the secretion rate of EFN in plant parts which face an increased risk of herbivory.

Keywords: Airborne signalling, ants, indirect defence, extrafloral nectar, herbivore-induced plant volatiles, herbivory, (3*Z*)-hex-3-enyl acetate, Lima bean, Mexico, plant-plant communication.

Introduction

A large number of plants respond to leaf damage with the induction of a variety of defences. Many of these defences are regulated via the octadecanoid pathway, with jasmonic acid acting as a central signalling molecule (CREELMAN & MULLET 1997; WASTERNACK & PARTHIER 1997). In addition to direct defences such as the production of leaf toxins, plants may also respond to herbivory with the emission of volatile chemicals that serve as host-location cues for carnivores (for review see PICHERSKY & GERSHENZON 2002; ARIMURA *et al.* 2005). Plant-mediated attraction of carnivorous arthropods to actively feeding herbivores is generally believed to function as an indirect defence that enhances the fitness of the volatile-emitting plant by increasing the predation pressure on the herbivores (TAKABAYASHI & DICKE 1996; PARÉ & TUMLINSON 1999).

Moreover, such plant volatiles provide chemical information about the status of attack of the emitting plant, which might be used not only by higher trophic levels (TURLINGS *et al.* 1995; KESSLER & BALDWIN 2001; VAN POECKE & DICKE 2004; ARIMURA *et al.* 2005), but also by neighbouring plants of the same or another species (BALDWIN & SCHULTZ 1983; ARIMURA *et al.* 2000a; KARBAN *et al.* 2000; FARMER 2001; KARBAN & MARON 2002).

Plants that can activate and tailor their defences accordingly to information derived from their neighbour may gain a selective advantage over plants that are unable to make use of this information. Whether plants positioned downwind are indeed capable of such con- or intra-specific 'eavesdropping' on airborne cues has been debated intensively (FOWLER & LAWTON 1985; SHONLE & BERGELSON 1995), yet evidence supporting such a plant-plant communication hypothesis is accumulating (e.g. BRUIN *et al.* 1992; KARBAN *et al.* 2000; TSCHARNTKE *et al.* 2001).

An increasing number of laboratory studies on different plant species suggest that plants can perceive volatile signals, as evidenced by transcriptional changes of defence-related genes (BATE & ROTHSTEIN 1998; ARIMURA *et al.* 2000b, 2001; GOMI *et al.* 2003; ARIMURA *et al.* 2005; KISHIMOTO *et al.* 2005; PASCHOLD *et al.* 2005; PENG *et al.* 2005). Furthermore, both laboratory and field studies have revealed that exposure to herbivore-induced (HI) volatile organic compounds (VOCs) results in changes in the abundance of phytohormones (ARIMURA *et al.* 2002; ENGELBERTH *et al.* 2004), as well as in an increasing production of defence-related metabolites such as terpenoids (ENGELBERTH *et al.* 2004; RUTHER & KLEIER 2005), proteinase inhibitors (FARMER & RYAN 1990; TSCHARNTKE *et al.* 2001) or phenolic compounds

(BALDWIN & SCHULTZ 1983; TSCHARNTKE *et al.* 2001).

Despite the number of laboratory studies that revealed such an aerial transfer of information between plants, relatively few studies report these effects under field conditions (e.g. FOWLER & LAWTON 1985; PRESTON *et al.* 2001). Especially evidence for the expression of defence-related plant metabolites after exposure to VOCs under field conditions is largely lacking (but see KARBAN *et al.* 2000).

Here we attempt to bridge this gap by identifying a new mechanism of plant-plant communication between herbivore-damaged and undamaged plants growing under natural conditions. To investigate this phenomenon, we chose Lima bean (*Phaseolus lunatus* L., Fabaceae), a model plant commonly employed in studies of induced defences. Besides the emission of HI-VOCs, Lima bean additionally features extrafloral nectaries that function as an indirect defence under natural conditions (KOST & HEIL 2005a), and whose nectar secretion rates are also inducible (HEIL 2004a). These traits make Lima bean an ideal system to investigate whether VOCs can induce another indirect defence trait (i.e. secretion of extrafloral nectar) in undamaged plants and whether this has fitness consequences for the receiver of the signal.

In field experiments performed at the plants' natural growing site we addressed the following questions: (1) Does the emission of HI-VOCs induce the secretion of extrafloral nectar (EFN) in undamaged, neighbouring tendrils? (2) Which compounds within the complex blend of HI-VOCs are responsible for the induction of EFN secretion? (3) Does the Lima bean benefit from a volatile-induced secretion of EFN under natural growing conditions, and (4) Are putative plant defenders (ants and wasps) attracted to VOCs and EFN?

Materials and methods

Study site and species

Field work was done in 2003 and 2004 during the transition from wet to dry season (October – December). All field experiments were performed on a native population of Lima bean growing in the coastal area near Puerto Escondido in the state of Oaxaca, Mexico. The plants investigated were growing under natural field conditions along dirt roads leading to extensively used pastures or plantations at sites, where previous experiments had been performed (HEIL 2004a; KOST & HEIL 2005a). Due to the tangled growth of Lima bean, it was not always possible to ensure that the bean tendrils used in the experiment belonged to one single plant individual.

All laboratory experiments were performed with potted plants which were grown from seeds derived from plants of our study sites. According to preliminary analyses with AFLP markers (M. HEIL, unpublished data), these plants belong to

the 'Mesoamerican genotype' (*sensu* GUTIÉRREZ SALGADO *et al.* 1995). Plants were grown in plastic pots with a diameter of 14 cm. Growing conditions were 23 °C, 60 % humidity, and 270 µE m^{-2} s^{-1} during a 14 h photoperiod. Experiments in the laboratory were performed with 6 week old plants (i.e. 7 - 8 leaf stage).

Emission of HI-VOCs

Experiment 1. We enclosed pairs of naturally growing, basifixed tendrils with five leaves in gauze bags (mesh size 0.5 mm) and supplied one of the two bags with herbivores that had been previously observed feeding on Lima bean. Herbivores involved were mainly *Cerotoma ruficornis* and *Gynandrobrotica guerreroensis* (both Chrysomelidae), Mexican bean beetle *Epilachna varivestis* MULSANT (Coccinellidae), a curculionid species as well as one species of both Ensifera and Caelifera. Abundance and species composition of the caged herbivores mirrored the frequency of occurrence during insect sampling and thus was a representative cross-section through the local herbivore community typically attacking Lima bean plants. Each gauze bag was supplied with 6 Ensifera or Caelifera as well as with 10 beetles of different species.

After 48 h, the naturally induced tendrils were detached, immediately supplied with a water reservoir and the herbivores carefully removed. Then, the tendrils were bagged in a PET foil ('Bratenschlauch', Toppits, Minden, Germany) that does not emit detectable amounts of volatiles by itself. The emitted VOCs were collected continuously over 24 h on charcoal traps (1.5 mg charcoal, CLSA-Filters, Le Ruissaeu de Montbrun, France) using air circulation as described previously (DONATH & BOLAND 1995). After 24 h, leaves were dried for dry mass determination and volatiles were eluted from the carbon trap with dichloromethane (40 µl) containing 1-bromodecane (200 ng $µl^{-1}$) as an internal standard. Samples were then transferred to glass capillaries, sealed by melting the open end and stored at < 5 °C for transport to Germany. Samples were analyzed on a GC-Trace mass spectrometer (Thermo Finnigan: www.thermofinnigan.com) according to KOCH *et al.* (1999). Individual compounds (peak areas) were quantified with respect to the peak area of the internal standard and related to the dry weight of the measured tendril.

Experiment 2. In a laboratory experiment we verified whether the detachment of Lima bean tendrils alters the qualitative and quantitative blend composition of the emitted HI-VOCs. Therefore, the five youngest leaves of two potted Lima bean plants were exposed to 5 adult *E. varivestis* for 2 d at room temperature, and two control plants remained undamaged. After 24 h, the herbivory rate of the two herbivore-damaged tendrils was estimated according to KOST and HEIL (2005a) to

assure that the mean leaf area consumed was similar between either tendrils (paired t-test: $P > 0.05$, $n = 9$). One damaged and one undamaged tendril were cut and supplied with a water reservoir and the two other plants remained potted. Volatiles were collected from the five youngest leaves of all four plants for the next 24 h as described for Experiment 1. This experiment was replicated nine times.

EFN collection: general procedure

The production rate of EFN was determined as amounts of secreted soluble solids (i.e. sugars, amino acids; see e.g. HEIL *et al.* 2000b), by quantifying the nectar volume with micro capillaries and the nectar concentration with a portable, temperature-compensated refractometer (HEIL *et al.* 2000b, 2001).

In a preceding laboratory experiment we verified whether the amount of secreted EFN depends on the dry weight of the secreting leaf. Therefore, the accumulated EFN of 30 potted Lima bean plants at the 7 - 8 leaf stage was washed off with pure water. Fourteen of these plants were sprayed with an aqueous solution of 1 mmol jasmonic acid (JA) until the surfaces of all leaves were covered, while the remaining 16 control plants remained untreated. After 24 h, the amount of newly secreted EFN of the five youngest leaves was quantified as described above and the leaves were dried for dry weight (DW) determination. Since the amount of secreted EFN depended strongly on the dry weight of the secreting leaves of both control (linear correlation $r = 0.811$, $n = 16$, $P < 0.001$) and JA-treated tendrils (linear correlation after reciprocal transformation $r = 0.57$, $n = 14$, $P < 0.05$), the measured amounts of EFN produced was related to the dry weight of the secreting leaves.

EFN-induction experiments

The amount of secreted EFN strongly depends on parameters such as light intensity (C. KOST, personal observation; HELDER 1958; MICHAUD 1990; PACINI *et al.* 2003) or leaf age (C. KOST, unpublished data; HEIL *et al.* 2000b). To reduce the variability caused by environmental influences (e.g. different light intensities), we used a paired experimental design with the maximum distance between two tendrils of each pair being < 1 m. The leaf-age as factor influencing the EFN secretion rate was taken into account by matching tendrils pairs of similar leaf age. Due to this experimental approach, not the absolute amount of EFN secreted, but the relative difference between treated and control tendrils is the appropriate measure to assess any given effect.

Tendrils used for all EFN-induction experiments were undamaged basifixed tendrils with five leaves which were placed in mesh bags (mesh size 0.5 mm). A ring of sticky resin (Tangletrap®, Tanglefoot Company, Grand Rapids, Michigan, USA) was applied at their base to protect the

tendrils from flying and crawling nectar consumers.

Experiment 3. Wild growing tendrils were exposed to herbivores naturally feeding on Lima bean as described above (*Experiment 1*). After 48 h, the naturally induced tendrils ('emitter tendrils') were abscised, immediately supplied with a water reservoir and the herbivores carefully removed. The detached emitter tendrils were then packed together with a basifixed bean tendril ('receiver tendril') into a perforated PET-plastic bag ('Braten-schlauch', Toppits, Minden, Germany). Receiver tendrils packed together with detached, undamaged emitter tendrils served as controls accordingly. The two tendrils (emitter and receiver tendril) were arranged in a way that the emitter tendril entered the plastic bag from above and the receiver tendril from below. Both ends were tied up with a cord with the water reservoir projecting out of the lower end of the assembly. EFN production rates of receiver tendrils were quantified after 24 h. The plastic bags used for this experiment were perforated with a conventional office hole-punch (hole area 0.78 cm^2) in regular intervals, resulting in a perforation ratio of 11 %. This ratio was a result of previous trials, where the number of punched holes was adjusted in a way that even after direct exposure to sunlight, the packed tendril did neither show any wilting symptoms nor any wetting at the inside of the foil within 24 h.

Experiment 4. A blend of synthetic VOCs was prepared and dissolved in lanolin paste (Sigma-Aldrich: www.sigma-aldrich.com) as a matrix, from which the volatiles could evaporate (KESSLER & BALDWIN 2001). According to preceding measurements, this mixture was adjusted to mimic the herbivore-induced bouquet both quantitatively and qualitatively as to emission within 24 h. The artificial blend consisted of 0.12 µg (*R*)-(-)-linalool, 0.13 µg β-caryophyllene, 0.19 µg methyl salicylate, 0.26 µg (*Z*)-jasmone (all purchased of Sigma-Aldrich), 0.02 µg (3*Z*)-hex-3-enyl acetate (Avocado Research Chemicals Ltd., Leysham, Lancaster, UK), 0.85 µg (*E*,*Z*)-β-ocimene (mixture of (*E*)-isomer (70 %) and (*Z*)-isomer (30 %) kindly provided by ROGER SNOWDEN, Firmenich, Geneva, Switzerland), 0.63 µg (3*E*)-4,8-dimethylnona-1,3,7-triene DMNT) and 0.9 µg (3*E*,7*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) (synthesized by standard methods (PATTENDEN & WEEDON 1968)) per µl lanolin. Purity of all compounds was > 98 %.

To verify whether the VOCs released from the lanolin paste mimicked the herbivore-induced bouquet also under field conditions, seven pairs of detached Lima bean leaves were supplied with green plastic strips on which 40 µl of either the artificial VOC mixture or lanolin only were spotted. The headspace of these leaves was sampled for 24 h as described for Experi-

ment 1. Use of the green plastic strips should prevent any diffusion of compounds into the plant.

Experiment 5. Lanolin paste containing the artificial volatile blend (40 µl) was applied to each of five leaves of a bean tendril and compared to a neighbouring control tendril to which lanolin paste only was applied. Both control paste and paste containing volatiles were spotted on green plastic strips attached to tendrils. The production rate of EFN was quantified after 24 h. This experiment has been conducted in 2003 (n = 10) as well as in 2004 (n = 12).

Experiment 6. Pairwise comparisons between tendrils which have been treated with lanolin alone and untreated tendrils allowed to test for an effect of lanolin on the EFN production rate. Sample size for this experiment was twelve pairs of tendrils.

Experiment 7. With the same experimental design as has been used in Experiment 5, we performed single-compound comparisons with all eight constituents of the complete blend. For this purpose we mixed blends that contained 1 µg of every compound per µl lanolin and 40 µl of this mixture were applied per leaf as described above. Sample sizes for this experiment are given in table 1.

Long-term experiment

Experiment 8. The aim of this experiment was to compare the defensive effect of EFN alone to the effect mediated by airborne volatiles (i.e. EFN induction and attraction of plant defenders). For this purpose, we used a paste containing volatiles which was similar to the one used in Experiment 4 and prepared an artificial EFN. Both mixtures were adjusted to mimic the natural production rates within three days both quantitatively and qualitatively as determined in previous experiments (C. KOST, unpublished data; KOST & HEIL 2005a). The artificial EFN consisted of an aqueous solution of 4.01 g sucrose l^{-1}, 24.24 g l^{-1} of each fructose and glucose, which are the main constituents of Lima bean EFN (KOST & HEIL 2005a).

Seventeen groups of four neighbouring tendrils each growing < 3 m apart from each other were selected as experimental units. All tendrils selected were trained along supporting ropes, and the tendrils of each group were randomly assigned to one of four treatments. Tendrils were either left untreated (control group) or treated every 3 d with lanolin paste only (treatment control group), artificial volatile blend (volatile group) or artificial nectar mixture (nectar group). The lanolin paste was applied as described above, whereas the EFN mimic (40 µl per trifoliate leaf) was applied directly to the extrafloral nectaries of every leaf of each tendril of the nectar group. To determine the effect of the four treatments on the fitness of the treated tendrils, we quantified the fitness-relevant plant parameters, herbivory rate (as percent leaf

loss) and the numbers of newly developed leaves and inflorescences. The number of leaves and inflorescences were quantified by counting and the herbivory rate as described in KOST and HEIL (2005a). All three parameters were quantified at the beginning of the experiment and after 25 d. The differences between these two values were calculated to determine the development of the respective parameter.

Experiment 9. The influence of our treatments on the presence of putative plant defenders was investigated by assessing the number of ants and wasps that visited the study tendrils. Fourteen censuses were performed within 3 d at two sites (at site 1 on day 7 and at site 2 on day 18 after the beginning of the experiment) and insect numbers were pooled for each tendril.

Statistical analyses

The high variability of the EFN secretion rates measured in Experiments 3, 5, 6 and 7, which is caused by environmental factors (see above), allowed comparisons between two groups of paired tendrils, yet no multiple statistical testing between different tendril pairs. These experiments were therefore considered independent and analysed by using paired t-tests without a multiple-comparison correction (such as e.g. Bonferroni). Differences in the emission rate of VOCs (Experiments 1, 2 and 4) were estimated using exact Mann-Whitney U tests and Wilcoxon signed rank tests for matched pairs. Despite deploying multiple comparisons to one data set, we did not use a multiple-comparison correction. By adjusting the type I error (i.e. error of incorrectly declaring a difference) downwards, such a procedure would have increased the chance of making a type II error (i.e. no difference is declared, while in fact there is a difference). Experiments 1, 2 and 4 aimed at comparing our treatments to the natural situation. Thus, accepting type I rather than type II errors was the more conservative approach.

Data of the long-term experiment (Experiments 8 and 9) were analysed by applying a mixed-effect model with 'treatment' as a fixed and 'tendril group' as a random factor. The number of leaves were ln-transformed and the number of inflorescences log-transformed to meet the assumption of homogeneity of variances. Post-hoc comparisons (LSD) were performed to test for statistically significant differences between treatments. Data were analysed using SPSS 13.0 (SPSS for Windows, SPSS Inc., Chicago, USA).

Results

Emission of HI-VOCs

In response to herbivore attack, Lima bean plants released a blend of VOCs comprised of eight main constituents (Experiments 1 and 2; Figs. 1 and 2). Detachment of bean tendrils neither changed the total amount of volatiles emitted (Wilcoxon

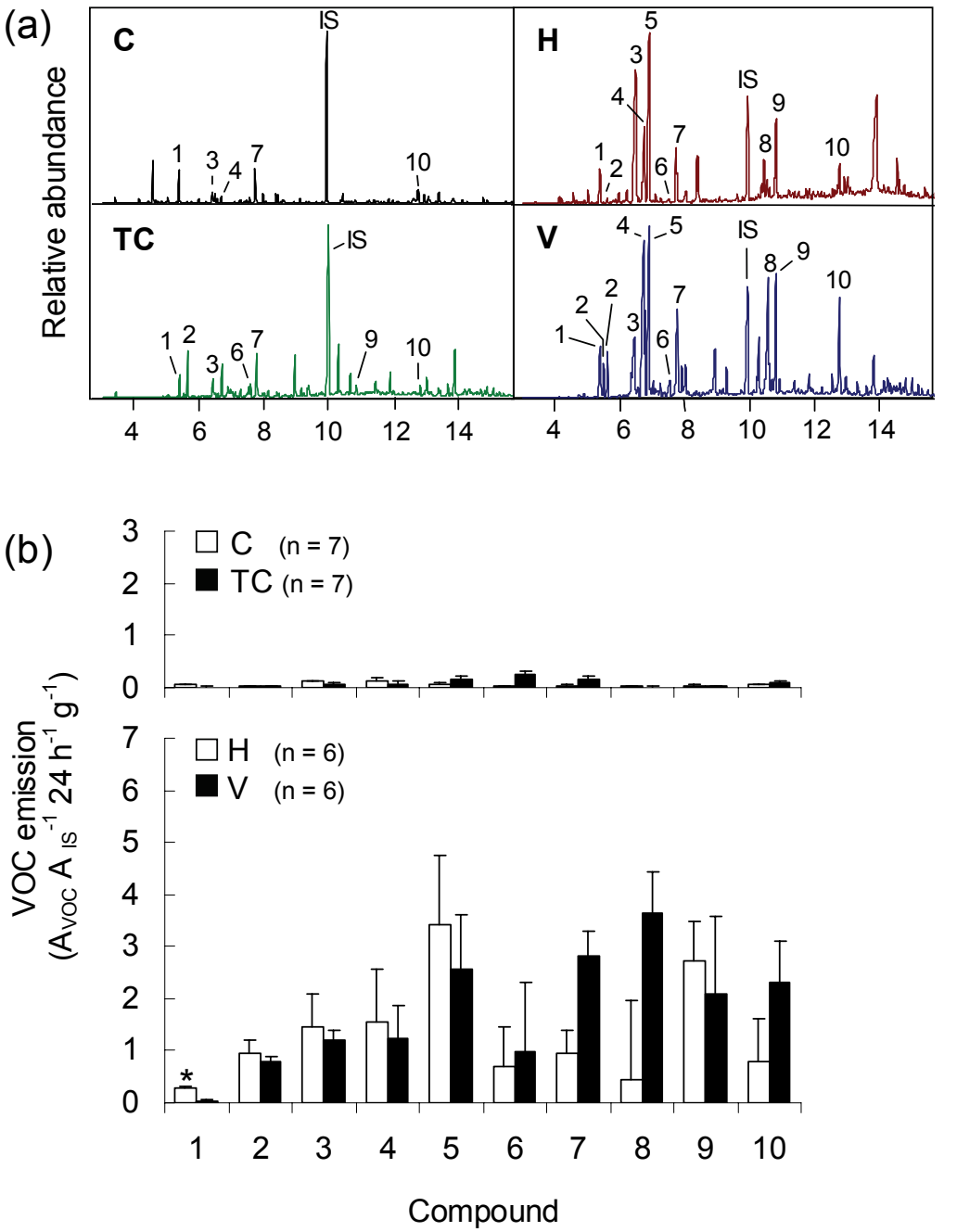


Figure 1. Volatile blends resulting from different treatments (Experiments 1 and 4). (**a**) Representative gas chromatographic profiles of an undamaged bean tendril (C), an herbivore-induced bean tendril (H), a single leaf treated with lanolin paste (TC) or a single leaf treated with artificial volatile blend dissolved in lanolin paste (V). (**b**) Pairwise comparisons between mean (+ SEM) VOC emission of undamaged tendrils (C,) and tendrils treated with lanolin paste (TC) as well as between herbivore-induced bean tendrils (H) and tendrils treated with the artificial volatile blend dissolved in lanolin paste (V). The amount of emitted VOCs is given as peak area relative to the peak area of an internal standard per 24 h and per g dry weight. Asterisks denote significant differences between C and CT as well as between H and V for every analysed VOC (exact Mann-Whitney U test, *, $P < 0.05$). Identified compounds are: 1, (3*Z*)-hex-3-enyl acetate ; 2, (*E*,*Z*)-β-ocimene; 3, (*R*)-(-)-linalool; 4, DMNT; 5, $C_{10}H_{14}$; 6, methyl salicylate; 7, $C_{10}H_{16}O$; 8, (*Z*)-jasmone; 9, β-caryophyllene; 10, TMTT; IS, internal standard (1-bromodecane).

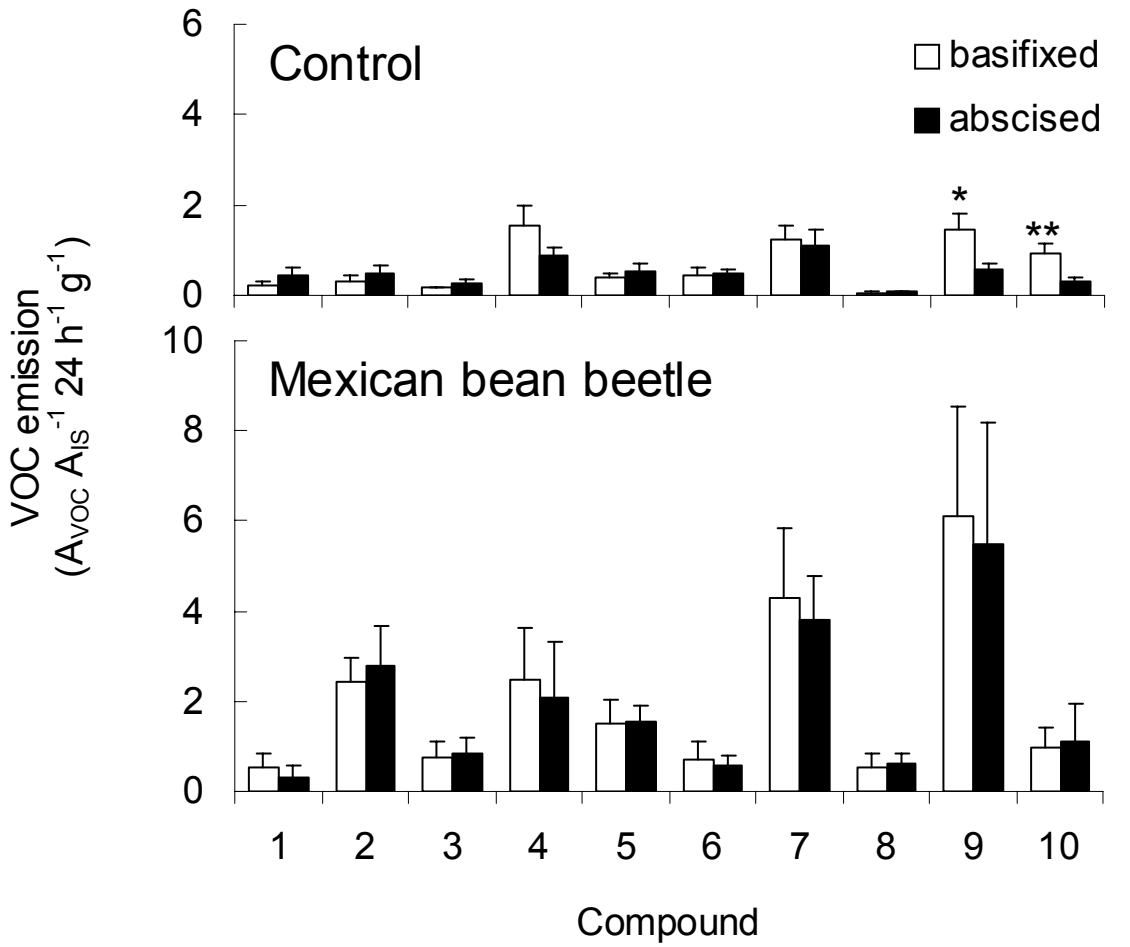


Figure 2. Comparison of the mean (+ SEM) VOC emission between basifixed and abscised tendrils from undamaged plants (Control) and plants damaged by five adult individuals of *Epilachna varivestis* (Mexican bean beetle) (Experiment 2). The amount of emitted VOCs is given as peak area relative to the peak area of an internal standard per 24 h and per g dry weight. The ten most dominant compounds are shown: 1, (3*Z*)-hex-3-enyl acetate; 2, (*E*,*Z*)-β-ocimene; 3, (*R*)-(-)-linalool; 4, DMNT; 5, $C_{10}H_{14}$; 6, methyl salicylate; 7, $C_{10}H_{16}O$; 8, (*Z*)-jasmone; 9, β-caryophyllene; 10, TMTT. Asterisks denote significant differences between basifixed and abscised tendrils for every analysed VOC (Wilcoxon signed rank test, *, $P < 0.05$; **, $P < 0.01$). Sample size was nine plants per treatment.

signed rank test: $P > 0.05$, $n = 9$), nor the emission rate of the eight main constituents of the blend in comparison to potted plants (Experiment 2; Fig. 2, Wilcoxon signed rank test for all eight compounds: $P > 0.05$, $n = 9$). This held true for both undamaged control plants and herbivore-damaged plants. Solely in undamaged plants, detachment of bean tendrils caused a significant decrease in the emission rate of β-caryophyllene and the homoterpene TMTT as compared to potted plants (Fig. 2, Wilcoxon signed rank tests: $P < 0.01$ and $P < 0.05$, $n = 9$).

EFN-induction experiments

To test whether HI-VOCs could induce the secretion of EFN in neighbouring plants,

we exposed basifixed receiver tendrils of wild growing plants to VOCs emitted from detached, herbivore-damaged emitter tendrils. Quantification of EFN after 24 h indicated that receiver tendrils exposed to naturally induced VOCs showed a two-fold increase in EFN secretion rate as compared to controls (Experiment 3; Fig. 3, paired t-test: $P < 0.001$, $n = 9$).

If airborne VOCs were responsible for the observed effect, then also synthetic compounds should be capable of inducing EFN secretion in undamaged plants. We tested this hypothesis by preparing an artificial volatile blend which we dissolved in lanolin paste. Volatile collection under field conditions confirmed that the artificial blend indeed mimicked the blend emitted from naturally induced tendrils both qualitatively and - to a large degree - quantitatively (Experiment 4; Fig. 1). However, the artificial VOC-mixture released significantly lower amounts of (3*Z*)-hex-3-enyl acetate than herbivore-damaged tendrils (Fig. 1, exact Mann-Whitney U test: $P < 0.05$).

Application of this artificial volatile blend to bean tendrils, and as a consequence thereof, an increased amount of

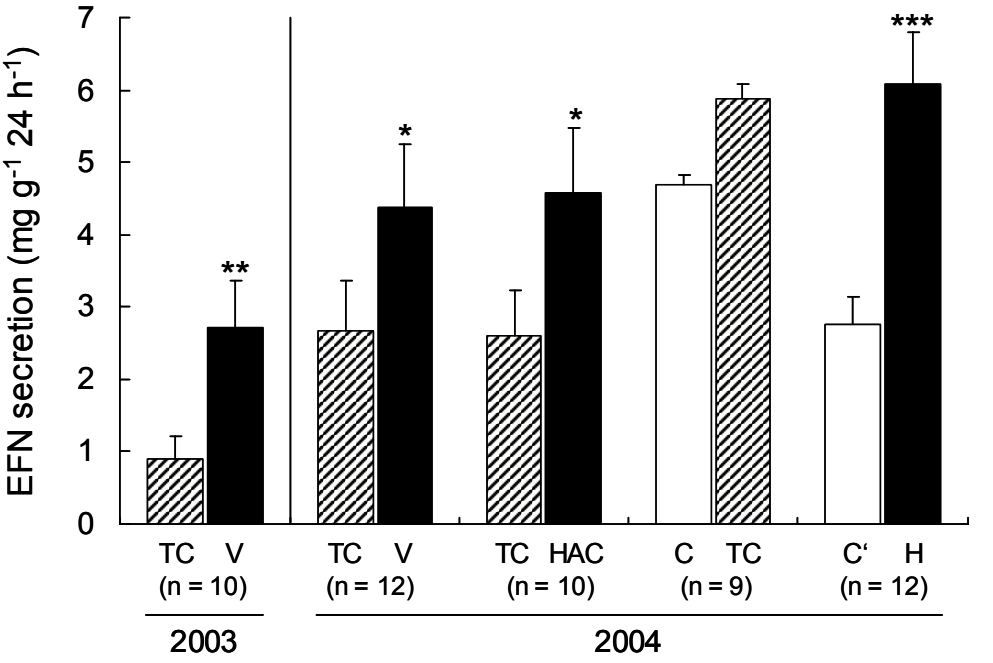


Figure 3. Extrafloral nectar (EFN) secretion in response to different treatments. Mean (+ SEM) EFN secretion rates are given in mg soluble solids per g leaf dry mass per 24 h. Pairwise comparisons between tendrils treated with lanolin paste (TC) and the artificial volatile blend (V) (Experiment 5) or (3*Z*)-hex-3-enyl acetate (HAC) dissolved in lanolin (Experiment 7) are depicted. Furthermore, the EFN secretion rate of untreated tendrils (C) and tendrils treated with lanolin paste (TC) (Experiment 6) as well as between tendrils exposed to detached, undamaged tendrils (C') and tendrils exposed to detached, herbivore-damaged tendrils (H) (Experiment 3) are displayed. Asterisks denote significant differences between pairs of tendrils (paired t-test, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Table 1. Extrafloral nectar (EFN) secretion from natural growing Lima bean tendrils after treatment with single constituents of the herbivore-induced volatile blend.

		EFN secretion rate (mg g^{-1} 24 h^{-1})		
Compound (No.)	**n**	**Control**	**Compound**	**P**
(3*Z*)-Hex-3-enyl acetate (1)	10	2.6 (± 0.6)	4.6 (± 0.9)	0.040
(*E*,*Z*)-β-Ocimene (2)	7	2.2 (± 0.8	2.5 (± 0.6	0.542
(*R*)-(-)-Linalool (3)	10	3.5 (± 0.9)	4.9 (± 0.9)	0.300
DMNT (4)	8	2.8 (± 0.9)	4.3 (± 0.8)	0.246
Methyl salicylate (6)	7	4.4 (± 0.7)	3.5 (± 0.4)	0.104
(*Z*)-Jasmone (8)	8	4.8 (± 0.7)	4.5 (± 0.7)	0.746
β-Caryophyllene (9)	6	3.9 (± 0.6)	3.1 (± 0.8)	0.296
TMTT (10)	8	4.7 (± 1.0)	6.3 (± 1.5)	0.405

Pairwise comparisons between wild growing, neighboring tendrils of five leaves, each treated with either 40 µl of lanolin paste (Control) or lanolin paste containing 1 µg $µl^{-1}$ of a single compound (Compound), are displayed. Number of compounds accord to Figures 2 and 3. Mean (± SEM) EFN secretion rates in mg soluble solids per g leaf dry mass per 24 h and P values of paired t-tests between both treatment groups are given.

VOCs in the headspace of Lima bean tendrils, significantly increased the EFN secretion rate of treated tendrils as compared to controls that had been treated with lanolin only (Experiment 5; Fig. 3, paired t-test: $P < 0.01$, $n = 10$). Originally performed in November 2003, a replication of this experiment in October 2004 validated the initial result (Fig. 3, paired t-test: $P < 0.05$, $n = 12$). A comparison of the EFN production rate between untreated tendrils and tendrils to which lanolin only had been applied revealed no statistically significant difference between either groups (Experiment 6; Fig. 3, paired t-test: $P > 0.05$, $n = 12$).

Which compounds of the applied volatile blend elicited the observed increase in EFN secretion? To answer this question, the EFN secretion rate of control tendrils (lanolin only) were pairwise compared with tendrils that had been treated with a lanolin mixture containing only one of the eight main constituents of the complete herbivore-induced blend (Experiment 7; Table 1). Among these compounds, only (3*Z*)-hex-3-enyl acetate significantly increased EFN secretion rates (Fig. 3, Table 1). Other constituents tested, for example (*R*)-(-)-linalool, DMNT and TMTT, showed a trend towards an inductive effect, yet caused no statistically significant increase in EFN secretion rate (Table 1). The strength of the EFN-induction effect of (3*Z*)-hex-3-enyl acetate resembled the one of the complete mixture of synthetic VOCs (Fig. 3).

Long-term experiment

This experiment was conducted to verify whether VOC-induced EFN secretion indeed enhances the defence status of wild growing Lima bean plants (Experiments 8 and 9). Therefore we compared the defensive effect of EFN alone to the volatile-mediated effect, i.e. attraction of plant defenders to VOCs and to VOC-induced EFN. After 25 d of repeatedly applying a synthetic mixture of EFN and VOCs (i.e. every 3 d), fitness-relevant plant parameters such as the number of inflorescences (Fig. 4, univariate ANOVA: $P < 0.01$, $n = 17$) and leaves (Fig. 4, univariate ANOVA: $P < 0.001$, $n = 17$) were significantly increased in the two treatment groups (nectar and volatiles) as compared to both control groups (control and treatment control) (Experiment 8). Moreover, tendrils treated with the artificial blends of VOCs and EFN had received significantly less damage by leaf-chewing herbivores (Fig. 4, univariate ANOVA: $P < 0.01$, $n = 17$) than did control tendrils. Remarkably, the intensity of the defensive effects observed in the nectar and the volatile group were quantitatively indistinguishable (Fig. 4).

The numbers of ants and wasps that visited the studied tendrils at the end of the experiment were significantly increased on ants and wasps: $P < 0.001$, $n = 17$) as compared to controls (Experiment 9; Fig. 5). Again, no statistically significant difference could be detected between nectar

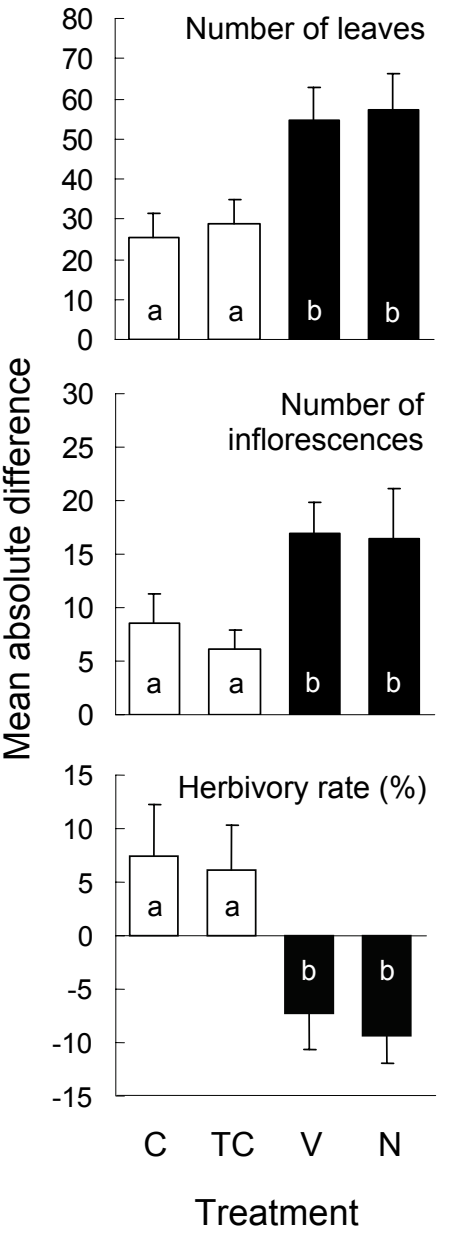


Figure 4. Effects of volatile and nectar treatment on fitness-relevant plant parameters (Experiment 8). Differences between measurements at t = 0 d and t = 25 d are displayed (mean + SEM). Groups of tendrils were left untreated (control, C), or were treated at regular intervals (3 d) with lanolin paste (treatment control, TC), artificial volatile blend dissolved in lanolin paste (volatiles, V) or an artificial extrafloral nectar (nectar, N). Different letters indicate significant differences among treatments (univariate ANOVA, $P < 0.05$ according to LSD post-hoc test). Sample size was seventeen groups of tendrils.

and volatile group. However, wasps were generally 25-times less frequently observed than ants (Fig. 5).

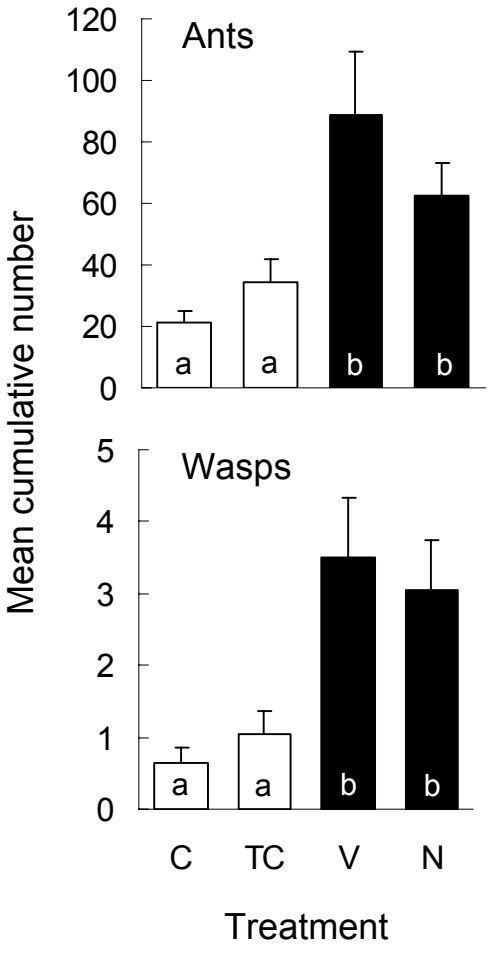


Figure 5. Effects of volatile and nectar treatment on insect numbers (Experiment 9). Insects, residing on bean tendrils that were left untreated (control, C), or were treated at regular intervals (3 d) with lanolin paste (treatment control, TC), artificial volatile blend dissolved in lanolin paste (volatiles, V) or an artificial mixture of extrafloral nectar (nectar, N), were counted. Fourteen censuses were performed within 3 d at two sites (site 1 on day 7 and site 2 on day 18 after the beginning of the experiment). Cumulated insect numbers (mean + SEM) are presented. Different letters indicate significant differences among treatments (univariate ANOVA, $P < 0.05$ according to LSD post-hoc test). Sample size was seventeen groups of tendrils.

Discussion

Our results demonstrate that HI-VOCs were recognized by undamaged, neighbouring Lima bean plants as a signal for an impending herbivore attack, which subsequently prompted them to activate their own defence, in our case the secretion of EFN. Both naturally emitted VOCs (Experiment 3) and a mixture of synthetic VOCs (Experiment 5) were capable of inducing EFN secretion in receiver tendrils. (3*Z*)-hex-3-enyl acetate was identified as a biologically active substance (Experiment 7) and the application of the mixture of synthetic VOCs benefited Lima bean under natural growing conditions (Experiments 8 and 9).

Contrasting the VOC profiles emitted from Lima bean plants, which had been attacked by either a mixture of natural folivores (Experiment 1; Fig. 1) or Mexican bean beetles *E. varivestis* (Experiment 2; Fig. 2) revealed quantitative rather than qualitative differences between the induced blends. The VOCs released from *P. lunatus* upon herbivore damage largely matched those of previous reports (DICKE *et al.* 1999; ARIMURA *et al.* 2000a; OZAWA *et al.* 2000; HEIL 2004a; MITHÖFER *et al.* 2005). However, it appears that the particular type of damage inflicted (chewing vs. cell-content feeding) has a major influence on the qualitative composition of the emitted VOC blend. Chewing herbivores, no matter if caterpillar, beetle or even snail provoke a similar blend of

VOCs (this study; MITHÖFER *et al.* 2005), whereas cell-content feeders such as mites frequently induce compounds which are absent in the VOC profile induced by chewing herbivores (DICKE *et al.* 1999; ARIMURA *et al.* 2000a). As a first step in investigating whether VOCs can induce EFN secretion, we focused our analysis on the eight most dominant compounds which are induced independently of the feeding mode of the attacking herbivore.

Although it is known that ablation of leaves or branches may alter the amount and blend composition of VOCs emitted from herbivore-infested plants (ARIMURA *et al.* 2001; SCHMELZ *et al.* 2001), we could experimentally demonstrate that the HI-VOC profiles emitted from abscised tendrils and basifixed plants were highly similar (Experiment 2; Fig. 2). This finding allowed us to use detached tendrils as VOC-emitters for our EFN induction experiment (Experiment 3).

Studying airborne communication between plants under field conditions is a daunting task: Plants in their natural environment are exposed to a multitude of additional signals, and other factors such as e.g. disturbing wind current may hamper the detectability of a given effect. This study intended to verify whether airborne VOCs can induce EFN secretion in Lima bean plants at all. Our approach, thus, was designed to increase the probability of detecting a respective effect under field conditions.

To reduce disturbing wind current and additional signalling from other VOC sources, we enclosed the receiver and the naturally induced emitter tendrils in bags of PET foil (Experiment 3). Previous studies (e.g. BATE & ROTHSTEIN 1998; ARIMURA *et al.* 2000a; TSCHARNTKE *et al.* 2001) were often criticised for using airtight chambers which may alter the physiological status of the enclosed plants by a shortage of CO_2 (BALDWIN *et al.* 2002; DICKE *et al.* 2003a). Therefore, we perforated the PET foil (perforation ratio 11 %) with relatively large holes of 0.78 cm^2. The enclosed tendrils did thus not show any symptoms of wilting or any wetting at the inside of the foil, even after exposure to direct sunlight. Our experimental conditions may have corresponded to the situation on a windless day. The observation that the artificial VOC mixture, which closely mimicked the naturally released blend (Experiment 5), was capable of inducing EFN secretion also in tendrils which were not enclosed at all, suggests a minor influence of the enclosure. However, future investigations in which Lima bean plants are exposed to herbivore-damaged neighbouring plants without any enclosure are necessary to clarify, whether this phenomenon occurs also under unconstrained environmental conditions.

The possibility of an information exchange between emitter- and receiver-tendrils via a systemic signal within the same plant individual (DICKE & DIJKMAN

2001; WÄCKERS *et al.* 2001) or via the rhizosphere between two different plant individuals (CHAMBERLAIN *et al.* 2001; PICKETT *et al.* 2003) seemed to be of minor importance in our experiments. Since we used detached emitter tendrils to expose basifixed receiver tendrils to the headspace of either undamaged or herbivore-damaged tendrils (Experiment 3), systemic plant responses as well as rhizosphere signalling could be excluded due to the missing physical connection between emitter- and receiver-tendrils. However, in the EFN-induction experiments with synthetic VOCs (Experiments 5 and 7), within-plant- or rhizosphere-signalling may have occurred. In this case, both mechanisms should have resulted in an induction of the control tendrils and thus to an underestimation of the observed effect. This line of reasoning suggests that the signal which elicited an increased EFN secretion has been transmitted aerially, rather than through the plant or the soil.

The use of synthetic VOCs allowed dissecting the full blend of HI-VOCs into its active constituents and thereby identification of (3*Z*)-hex-3-enyl acetate as eliciting the observed defence induction (Experiment 7; Table 1). (3*Z*)-hex-3-enyl acetate belongs to the group of so-called green leaf volatiles (GLVs), which are known to be emitted rapidly after leaf-damage (LOUGHRIN *et al.* 1994; TURLINGS *et al.* 1995; ARIMURA *et al.* 2000a), but are also produced *de novo* in response to herbivore damage by many plant species (ARIMURA *et al.* 2000a; KALBERER *et al.* 2001; KESSLER & BALDWIN 2001; RÖSE & TUMLINSON 2004).

(3*Z*)-hex-3-enyl acetate has been previously described to induce defence genes in uninfested leaves of Lima bean (ARIMURA *et al.* 2001) and *Arabidopsis* (BATE & ROTHSTEIN 1998) and to prime corn plants against subsequent herbivore damage (ENGELBERTH *et al.* 2004). An increased emission rate of (3*Z*)-hex-3-enyl acetate directly after mechanical wounding (i.e. within the first hour) of Lima bean plants (ARIMURA *et al.* 2000a) may facilitate a fast induction of the EFN secretion in neighbouring tendrils and thus allow to rapidly respond to a current threat.

Since (3*Z*)-hex-3-enyl acetate has been identified as biologically active substance, the decreased amounts of this GLV emitted from the mixture of synthetic VOCs (Fig. 1) should have led to an underestimation of the inductive effect and could explain the weaker effect of VOCs emitted from the synthetic VOC blend compared to herbivore-damaged bean tendrils (Fig. 3). The fact that the synthetic VOC mixture was capable of inducing the EFN secretion rate despite these decreased amounts of (3*Z*)-hex-3-enyl acetate (Fig. 1) indicates that also the other constituents of the synthetic VOC mixture may have contributed to EFN induction. Compounds that have been identified in other studies as eliciting VOC-induced plant responses, but which

did not show an effect on the secretion rate of EFN in this study, involve (Z)-jasmone (BIRKETT *et al.* 2000; BRUCE *et al.* 2003), (*E*,*Z*)-β-ocimene, DMNT, TMTT (ARIMURA *et al.* 2000a), and methyl salicylate (SHULAEV *et al.* 1997). Our experimental results neither exclude an inductive effect of a single, nor the combined action of several of these other blend constituents or compounds, which have not been detected with the applied analytical methodology for headspace sampling and VOC analysis.

Few studies conducted under field conditions clearly demonstrated fitness consequences for the receiver of the volatile signals (DOLCH & TSCHARNTKE 2000; KARBAN *et al.* 2000; KARBAN & MARON 2002). To verify whether VOC-induced EFN secretion enhances the defence status of Lima bean plants and thus plant fitness, we treated tendrils repeatedly (every 3 d) with the artificial VOC mixture (Experiment 8). Already after 25 d, these tendrils had lost less leaf area to herbivores and had produced more leaves and inflorescences than controls (Fig. 4). These parameters positively affect plant fitness. In a previous study conducted at the same sites, Lima bean plants that had been repeatedly induced with the chemical elicitor jasmonic acid suffered from less herbivore damage and showed a significant increase in the number of leaves, inflorescences, and fruits (HEIL 2004a). The increased number of inflorescences observed in this study did likely translate into an increased seed set and thus enhance the Lima bean's reproductive success.

Since VOCs induced EFN secretion, the tendrils of the volatile group experienced the combined defensive effect of both EFN and VOCs. Interestingly, the performance of all three fitness-relevant plant parameters measured in the volatile and the nectar group developed similarly (Fig. 4), i.e. no statistically significant difference could be detected. EFN obviously played a more important role as indirect defence in our study system than did volatile chemicals. VOCs may thus have rather functioned as EFN-inducing signals among bean tendrils located within a patch of increased herbivore pressure than as long-distant cues for flying plant defenders. However, our experimental approach does not exclude a protective effect exerted by volatile-attracted defenders. This issue should be addressed in future studies.

The growth architecture of the Lima bean at its natural growing site is characterized by (i) short distances among individual bean tendrils and (ii) a tangled growth that creates microenvironments with reduced wind current. Therefore, a systemic defence-inducing signal which is transported within the plant would be very ineffective (JONES *et al.* 1993). In contrast, an airborne signal would create a gradient of the infochemical around the site of attack, to which parts of the emitting plant or neighbouring plants can respond. Moreover, the intricate growth structure may

minimize dilution of airborne signals (THISTLE *et al.* 2004) and thus favour plant-plant communication.

Regarding the widespread taxonomic distribution of both extrafloral nectaries (KOPTUR 1992) as well as the capability of plants to respond to herbivore damage with the emission of VOCs (VAN POECKE & DICKE 2004), such a plant-plant communication response may represent a common mechanisms which adds a new facet to our understanding of the complex interactions among different trophic levels. Thus, the elucidation of this mechanism opens up new avenues for further studies that range from the underlying signalling cascades to the ecological relevance of this mechanism in our as well as in other study systems.

Acknowledgements

We thank S. ADOLPH and M. MEKEM SONWA for help with organic synthesis. We also thank I. T. BALDWIN, W. BOLAND, J. COLANGELO-LILLIS, R. DIETRICH, G. POHNERT, H. MAISCHAK, A. MITHÖFER, B. SCHULZE, T. WICHARD, W. W. WEISSER and four anonymous referees for critically reading earlier versions of the manuscript. W. BOLAND is gratefully acknowledged for the possibility to use all the facilities of the MPI. This study was financially supported by the German Research Foundation (DFG grants HE 3169/2-1,2,3,4).

Manuscript IV

The defensive value of two indirect defences of Lima bean in nature

Christian Kost[1]* and Martin Heil[1,2]

In preparation for *Oecologia*

[1] Department of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Beutenberg Campus, Hans-Knöll-Str. 8
D-07745 Jena, Germany

[2] Present address:
Department of General Botany
University Duisburg-Essen
FB BioGeo - Botanik
Universitätsstr. 5
D-45117 Essen, Germany

*** Corresponding author:**
Christian Kost
Department of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Beutenberg Campus
Hans-Knöll-Str. 8
D-07745 Jena, Germany
Phone: + + 49 - 3641 - 57 18 20
Fax: + + 49 - 3641 - 57 12 02
E-mail: ckost@ice.mpg.de

Abstract

The Lima bean (*Phaseolus lunatus*) features two indirect anti-herbivore defences - emission of volatile organic compounds (VOCs) and secretion of extrafloral nectar (EFN) - which are both inducible upon herbivore damage. In a previous study, Lima bean benefited of a simultaneous induction of both defences, yet it remained unclear whether both defences had contributed to plant protection. Our experimental approach aimed at separating the defensive function of both indirect defences. Tendrils sprayed with jasmonic acid (JA) to induce both indirect defences were compared to tendrils treated with an artificial blend of either EFN or VOCs as well as to control tendrils. Repeated application of EFN or VOCs benefited the treated tendrils as indicated by increased numbers of leaves, inflorescences and living shoot tips as well as a decreased herbivory rate and reduced numbers of dead shoot tips as compared to controls. Tendrils treated with JA showed a similar trend, yet some fitness parameters responded weaker to this treatment. This suggests that a putative JA-dependent direct defence was of minor importance, since otherwise JA-treated tendrils should have performed better than VOC- and EFN-treated tendrils. Comparing the amount of newly produced EFN between tendrils treated with JA and the artificial VOC mixture revealed that VOCs had induced EFN secretion in our experimental tendrils quantitatively similar to the JA treatment. Tendrils treated with synthetic EFN only, benefited equally or more of this treatment than did tendrils treated with VOCs or JA. Furthermore, field observation revealed that mainly ants and to a lower degree also wasps were significantly more attracted to tendrils treated with JA, VOCs and EFN than to control tendrils and were thus most likely responsible for the observed beneficial effect. Obviously, under our experimental conditions, EFN played a more important role as an indirect defence for Lima bean than a VOC-mediated arthropod attraction.

Key words: Ants, indirect defence, extrafloral nectar, plant-plant communication, volatile organic compounds.

Introduction

Plants respond to herbivore attack with a bewildering array of changes in plant chemistry, morphology, and physiology that frequently result in an increased resistance of plants to further attack (KARBAN & BALDWIN, 1997). Induced resistance may be due to direct effects on the herbivore with the plant producing toxic metabolites or anti-digestive and anti-nutritive compounds. Furthermore, plants may utilize indirect defences that facilitate ”top-down” control of herbivore populations by the herbivore's predators and parasitoids.

One way for a plant to attract beneficial arthropods is by providing suitable food sources such as extrafloral nectar (EFN; for review see KOPTUR, 1992). EFN is an aqueous solution with sugars and amino acids being the most abundant solutes (GALETTO & BERNARDELLO, 1992; HEIL *et al.*, 2000b; RUFFNER & CLARK, 1986), which is secreted from specialized organs, the so-called extrafloral nectaries. Additionally, plants may increase the emission rate of volatile organic compounds (VOCs) in response to herbivore attack that serve as cue guiding foraging parasitoids and predators to the feeding herbivore (PARÉ & TUMLINSON, 1997; TURLINGS *et al.*, 1990). The composition of the herbivore-induced volatile blend depends not only on the plant species or cultivar, but also varies with the species and even the larval stage of the herbivore (for review see ARIMURA *et al.*, 2005), thus providing highly reliable signals to the members of the third trophic level.

In many cases, plants do not only rely on one single defence strategy, but use a complex array of different defensive mechanisms. For example, several plant species have been identified that feature both VOC emission and EFN secretion (ARIMURA *et al.*, 2005). The presence of two indirect defences within one plant individual gives rise to the questions whether or not both defences contribute to plant fitness and how they interact in attracting beneficial arthropods (PRICE *et al.*, 1980). Studies on the benefit of indirect defence traits in nature are generally rare (HEIL, 2004a; KESSLER & BALDWIN, 2001; THALER, 1999) and no study to date has tried to disentangle the defensive value of two defences within one plant species.

The Lima bean (*Phaseolus lunatus*) is a common model system in studies on induced indirect defences. Recently, Heil (2004a) could demonstrate that wild growing Lima bean plants which have been induced by exogenous application of the phytohormone jasmonic acid (JA) responded with an increase of both the emission of VOCs and secretion of EFN. Repeated application of JA (i.e. every 3 d) resulted in a benefit for the treated plants as reflected e.g. by a decreased herbivory rate and an increased seed set. Further-

more, KOST and HEIL (2005a) demonstrated that artificially increasing the amount of available EFN benefits Lima bean in nature by attracting predacious and parasitoid arthropods. Thus, these two pilot studies represent an ideal starting point to unravel whether both induced defence traits contribute to plant defence or whether solely EFN secretion is responsible for Lima bean protection.

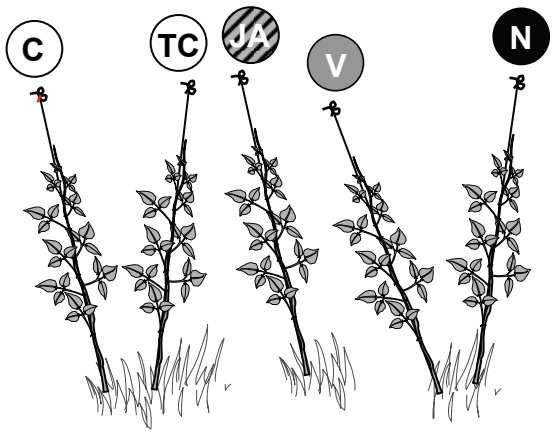


Figure 1. Experimental design. Five groups of Lima bean tendrils (*Phaseolus lunatus*) served as experimental unit with **C**, no treatment; **TC**, application of lanoline paste; **JA**, spraying with jasmonic acid; **V**, application of an artificial volatile blend dissolved in lanoline paste, and **N**, application of a artificial mixture of EFN. See Table 1 for details.

Therefore we chose an experimental approach similar to the one used in HEIL (2004a): Groups of five tendrils were used as experimental units (Fig. 1, Table 1). Within each group, two tendrils were either treated with JA or left untreated. The inclusion of two further bean tendrils in which the amount of either EFN or VOCs was artificially increased, enabled us to experimentally separate the protective effect of the two indirect defences. A fifth group served as a treatment control.

Repeated application of these five treatments allowed to answer the following questions: (i) What is the relative contribution of the two indirect defences - extrafloral nectar secretion and volatile emission - to the overall herbivore defence of the

Table 1. The five treatments and their effect on the treated tendrils.

Abbreviation	Treatment	Effect
C (Control)	no treatment	untreated control
TC (Treatment control)	application of lanolin paste	effect of lanolin paste
JA (JA-treatment)	spraying with jasmonic acid	induction of VOCs, EFN and putative JA-dependent direct defences
V (Volatile treatment)	application of a volatile mixture dissolved in lanolin paste	induction of EFN, attraction of arthropods to VOCs and EFN
N (Nectar treatment)	application of an artificial mixture of EFN	attraction of arthropods to EFN

Lima bean?, (ii) Is there an additional influence of a putative JA-dependent direct defence?, and (iii) What kinds of plant defenders are attracted to the treated tendrils?

Material and Methods

Study site and species

This study was conducted in the coastal area near Puerto Escondido in the state of Oaxaca, Mexico. The climate in the study area is characterized by one main rainy season from June to October, which follows a bimodal distribution peaking in July and September. Annual rainfall averages between 1 and 1.4 m and the mean annual temperature is 28 °C (STRÄßNER, 1999). Two sites were selected, which have already been used in previous studies (HEIL, 2004a; KOST & HEIL, 2005a). These two sites were located 15 km northwest of Puerto Escondido and about 3 km apart from each other. Here, Lima bean grows naturally along dirt roads leading to extensively used pastures or plantations. All experiments were performed on this native population of Lima bean plants. Field work was done in 2003 and 2004 during the transition from wet to dry season (October - December).

Experimental design

To reduce the variability that is caused by effects of site and genotype, 23 groups of five Lima bean tendrils growing < 3 m apart from each other were selected as experimental unit (Fig.1). Eleven groups were located at site 1 and 12 at site 2. Due to the tangled growth of Lima bean it was not always possible to ensure that all five tendrils of every group belonged to one single plant individual. All selected tendrils were trained along supporting ropes, and the tendrils of each group were randomly assigned to one of five treatments: Tendrils were either left untreated (control group) or treated every three to four days with lanolin paste only (treatment control group), sprayed with a aqueous solution of JA (JA group), an artificial VOC blend (VOC group) or an artificial mixture of EFN (EFN group) (Table 1). Starting 27th October 2003, the experiment lasted 25 d resulting in a total of six application events. During this time, the initial number of tendril groups was reduced to a final sample size of 17 due to cattle and human impact.

Treatment of tendril groups

The tendrils of the JA group were sprayed with an aqueous solution of 1 mmol JA until the surfaces of all leaves were covered. The artificial volatile blend and the synthetic mixture of EFN were adjusted to mimic its natural model within three days post-induction. Therefore, previous experiments had been performed to determine the qualitative and quantitative composition of the secreted EFN after induction with JA (KOST & HEIL, 2005a) and of

the volatile blend after herbivore feeding (KOST & HEIL, 2005b). The applied artificial EFN thus consisted of an aqueous solution of 4.01 g sucrose l^{-1}, 24.24 g l^{-1} of each fructose and glucose. 40 µl of this blend were applied with an Eppendorf pipette directly to the extrafloral nectaries of every trifoliate leaf of the EFN group.

The artificial volatile blend consisted of 0.12 µg (*R*)-(-)-linalool, 0.13 µg β-caryophyllene, 0.19 µg methyl salicylate, 0.26 µg (*Z*)-jasmone (all purchased of Sigma-Aldrich), 0.02 µg (*Z*)-3-hex-3-enyl acetate (Avocado Research Chemicals Ltd., Leysham, Lancaster, UK), 0.85 µg (*E*,*Z*)-β-ocimene (mixture of *E/Z*-isomers ca. 70:30; kindly provided by Roger Snowden, Firmenich, Geneva, Switzerland), 0.63 µg (3*E*)-4,8-dimethyl-nona-1,3,7-triene (DMNT) and 0.9 µg (3*E*,7*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) (synthesized by standard methods (PATTENDEN & WEEDON, 1968)) per µl lanolin. Purity of all compounds was > 98 %. Pure lanolin and lanolin paste containing volatiles were spotted on green plastic strips attached to tendrils to prevent any diffusion of compounds into the treated plants. 120 µl of both pastes were applied per five leaves of either volatile- or treatment control-group.

EFN-induction experiment

Our previous work suggested that herbivore-induced VOCs can induce the secretion of EFN in undamaged bean tendrils (KOST & HEIL, 2005b). Therefore, the aim of this experiment was to quantitatively compare the amount of EFN secreted from tendrils after exposure to airborne VOCs with the secretion rate of JA-induced tendrils. Eleven groups of three tendrils spread across the two sites were selected, which were located < 1 m apart. Each selected tendril was basifixed and had 5 leaves. The first tendril within each group was treated with lanolin paste, the second with lanolin paste containing volatiles and the third was sprayed with JA. All treatments were similar to the ones described above. The three tendrils were then placed in gauze bags (mesh size 0.5 mm) and a ring of sticky resin (Tangletrap®, Tanglefoot Company, Grand Rapids, Michigan, USA) was applied at their base as a protection against flying and crawling nectar consumers. After 24 h the amount of newly produced EFN was measured as the amount of secreted soluble solids (i.e. sugars, amino acids; see HEIL *et al.*, 2000b) by quantifying the nectar volume with micro capillaries and the nectar concentration with a portable, temperature-compensated refractometer (HEIL *et al.*, 2000b; HEIL *et al.*, 2001).

Fitness-relevant plant parameters

To assess the effect of our treatments on the fitness of the study tendrils, the following fitness-relevant plant parameters were considered: Number of leaves, inflorescences, living and dead shoot tips as well

as herbivory rate. The herbivory rate was estimated as percent leaf loss as described in KOST and HEIL (2005a), and the remaining parameters were quantified by counting. All five parameters were assessed at the beginning of the experiment and after 25 d. The differences between these two values were calculated to determine the development of the respective parameter in the course of the study period.

Insect counts and sticky traps

The insect community visiting the treated bean tendrils was assessed by counting and with sticky traps. Two series of insect countings were performed in which twenty groups of tendrils at both study sites were repeatedly visited. The first series of countings started at day 7 and the second on day 18 after the onset of the experiment. Ten groups of tendrils were selected at each study site and the number of ants, wasps or flies present on the plants were recorded, as they represented the most abundant groups. The first census was performed prior tendril treatment at 8:00 am. Thereafter, all tendrils were treated and insects on all pairs were counted repeatedly every 2 or 3 h until midnight. Two additional censuses were performed at 9:00 and 10:00 am on the following 2 days, resulting in a total of 14 monitorings. The number of all insects counted per experimental tendril was cumulated to test for an effect of the treatment on the number of insects observed.

To assess the functional groups of insects attracted to the experimental tendrils, two sticky traps were attached with plastic strings to each tendril of 14 groups, which were equally distributed between the two study sites. The sticky traps consisted of 100 cm^2 pieces of green plastic foil that had been coated with a thin layer of a trapping adhesive (Tangletrap®). After 24 h of exposure, the traps were recollected and the insects transferred to 75 % ethanol. Insects were identified to order or family level using keys and information provided by ARNETT (2000), SCHAEFER *et al.* (1994) and NOYES (2003). On the basis of the natural history information provided by HONOMICHL *et al.* (1996), DALY *et al.* (1998), KELSEY (1969; 1981) and MATILE (1997), the collected arthropods were assigned to the following guilds according to nutritional or functional aspects: Predator/entomophaga (R), parasitoid (P), utilization of plant-derived resources including floral or extrafloral nectar, pollen and honeydew (S), frugivore (F), herbivore and flower feeder (H), detrivore including phytosaprophage and zoosaprophage (D), blood-sucking and ectoparasitic (B), and fungivore (M).

Statistical analysis

Our randomized complete block design allowed analysing the data of the EFN-

induction experiment, the fitness-relevant plant parameters and the cumulative insect numbers with a mixed-effect model (univariate GLM procedure) with 'treatment' as fixed and 'tendril group' as random factor. The following variables have been transformed to meet the assumption of homogeneity of variances (transformation in brackets): number of living shot tips and number of wasps (square root), number of inflorescences and dead shoot tips (log), number of leaves, cumulative number of ants and flies (ln). Post-hoc comparisons (LSD) were performed to test for statistically significant differences between treatments. All statistical analysis was done using SPSS 13.0 (SPSS for Windows, SPSS Inc., Chicago, USA).

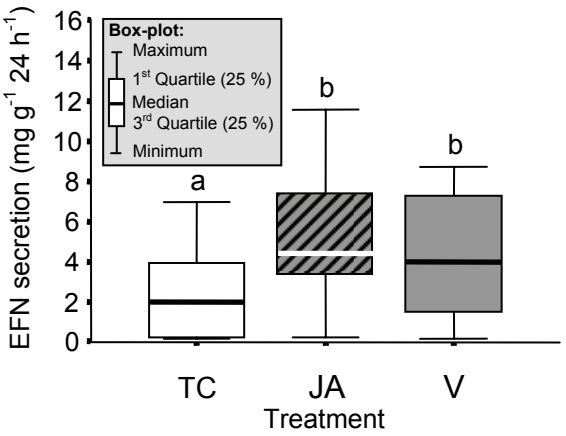


Figure 2. Effect of different treatments on the secretion rate of extrafloral nectar (EFN) given in mg soluble solids per g leaf dry mass per 24 h. The comparisons between tendrils treated with lanolin paste (**TC**), the artificial volatile blend dissolved in lanolin (**V**), and jasmonic acid (**JA**) are displayed. Different letters indicate significant differences among treatments (univariate ANOVA, $P < 0.05$ according to LSD post-hoc test). Sample size was eleven groups of tendrils. See insert for an explanation of box-whisker plots.

Results

Nectar-induction experiment

Treatment of bean tendrils with either the artificial volatile mixture dissolved in lanolin or with JA had a significant effect on the EFN secretion rate within 24 h as compared to controls treated with lanolin only (univariate ANOVA: $P < 0.01$, $n = 11$). Both treatments doubled the EFN secretion rate compared to the treatment control (Fig. 2). However, no statistically significant difference could be detected between the volatile and the JA-treated group. Thus, in the long-term experiment, tendrils of the JA and the volatile group had produced comparable amounts of EFN.

Effect of the treatments on fitness-relevant plant parameters

Already after 25 d, our three treatments JA, volatiles, and EFN had significant effects on the measured vegetative and reproductive plant traits of the treated tendrils: Tendril groups treated with volatiles and EFN showed a significant increase in the numbers of newly produced leaves, shoot tips and inflorescences, bore less dead shoot tips, and had suffered of less herbivore damage than the two controls (Fig. 3). JA-treatment also significantly decreased the number of dead shoot tips and the herbivory rate suffered as compared to the two control groups, yet no

such difference could be detected for the number of living shoot tips, leaves, and inflorescences. According to a LSD post-hoc test for these three parameters, the tendrils of the JA group took an intermediate position between EFN and volatile group on one hand and the two controls on the other side (Fig. 3).

Effect of the treatments on insect abundance

Ants, wasps and flies were the most abundant groups visiting the study tendrils. Among them, ants were the most dominant group: Comparing the number of ants with those of wasps and flies observed among the different treatments reveals that the number of ants counted on the experimental tendrils exceeded the number of flies 5 - 15-fold and the number of wasps even 20 - 30-fold (Fig. 4).

The five treatments significantly affected the insect visitation rate of the experimental tendrils. The mean cumulative

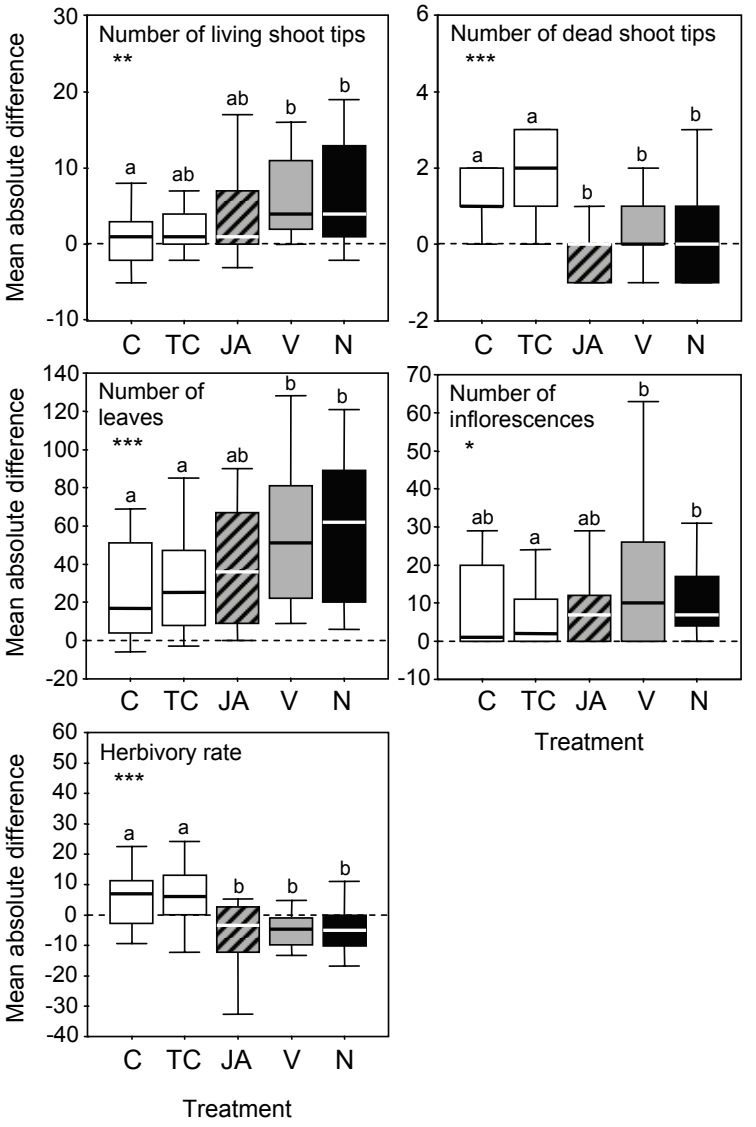


Figure 3. Effect of the five treatments on fitness-relevant plant parameters. Each tendril within groups of five Lima bean tendrils received one of five treatments: **C**, no treatment; **TC**, treatment with lanoline paste; **JA**, spraying with jasmonic acid; **V**, application of an artificial volatile blend dissolved in lanoline paste and **N**, application of an artificial mixture of EFN. See Tab.1 for details. Mean absolute differences between day 0 and day 25 of the experiment are displayed. All values above zero (dashed line) indicate an increase, all values below a decrease in comparison to the starting situation. Asterisks indicate significant treatment effects (univariate ANOVA, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) and different letters indicate significant differences among treatments (LSD post-hoc test, $P < 0.05$). Sample size was twenty groups of tendrils. See insert in Fig. 2 for an explanation of box-whisker plots.

ant number was significantly increased on tendrils treated with JA, volatiles and EFN as compared to the two control tendrils (Fig. 4, LSD post-hoc test after univariate ANOVA, $P < 0.05$). Volatile treatment doubled the median cumulative number of ants on experimental tendrils, while the JA and nectar treatment even lead to a three-fold increase compared to the untreated state. Correspondingly, median wasp number ranged between 1.5 (JA group), 2 (volatile group) and 2.5 (EFN group), while these insects were rarely encountered on control tendrils (Fig. 4). This difference between treatments was also significant (LSD post-hoc test after univariate ANOVA, $P < 0.05$). Flies responded weaker to the treatment of the experimental tendrils. Despite a significantly increased visitation rate of flies to JA-treated tendrils as compared to controls (Fig. 4, LSD post-hoc test after univariate ANOVA,

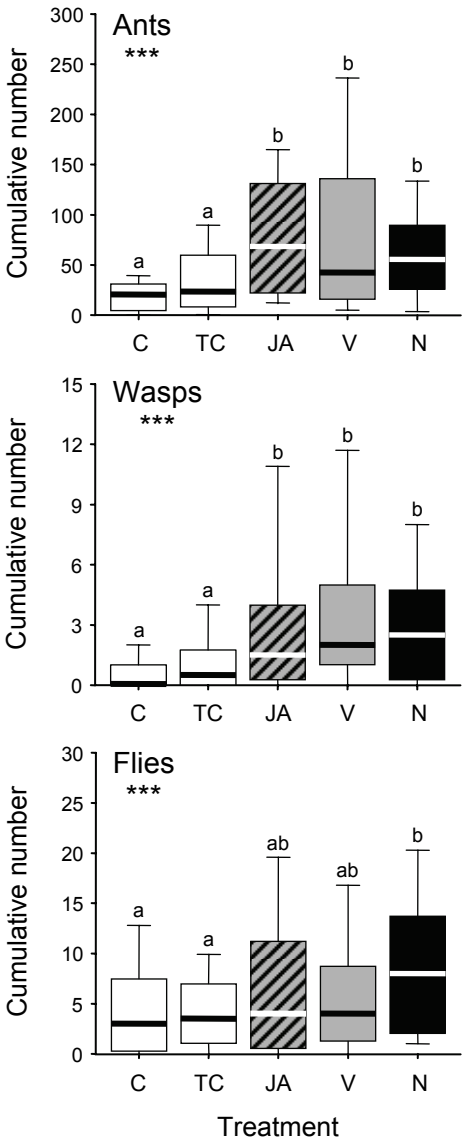


Figure 4. Effect of the five treatments on insect numbers visiting the Lima bean. Insects residing on Lima bean tendrils that received one of five treatments were counted. Treatments were: **C**, no treatment; **TC**, treatment with lanoline paste; **JA**, spraying with jasmonic acid; **V**, application of an artificial volatile blend dissolved in lanoline paste and **N**, application of an artificial mixture of EFN. See Tab. 1 for details. Fourteen censuses were performed within 3 d at two sites (site 1 on day 7 and site 2 on day 18 after the beginning of the experiment). Insect numbers were pooled per counted tendril. Asterisks indicate significant treatment effects (univariate ANOVA, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) and different letters indicate significant differences among treatments (LSD post-hoc test, $P < 0.05$). Sample size was twenty tendril groups. See insert in Fig. 2 for an explanation of box-whisker plots.

P < 0.05), no such effect could be observed for VOC- and EFN- treated tendrils (Fig. 4). The two latter groups were statistically indistinguishable from the JA-treated tendrils and both control groups.

Community composition of arthropods visiting Lima bean

A total number of 899 arthropods were caught on the sticky traps and > 94 % could be identified to the order or family level. Among them, Diptera (55 %) and Hymenoptera (26 %) were the most abundant insect groups captured (Table 1, Fig. 5). Other groups trapped were mainly Coleoptera (6 %), Araneida (5 %) and Thysanoptera (3 %). Interestingly, 73 % of all trapped arthropods were characterized by parasitoid or predacious life habits and may thus be classified as potentially beneficial to the Lima bean (Fig. 5). On the other hand, only 16 % of the trapped arthropods were herbivores or flower feeders and therefore potentially detrimental to the plant. Other plant-derived food sources like EFN, pollen or honeydew are known to be used by 67 % of all trapped arthropods and 19 % of all trapped taxa rely on other food sources such as e.g. fungi, fruits or detritus. The two latter

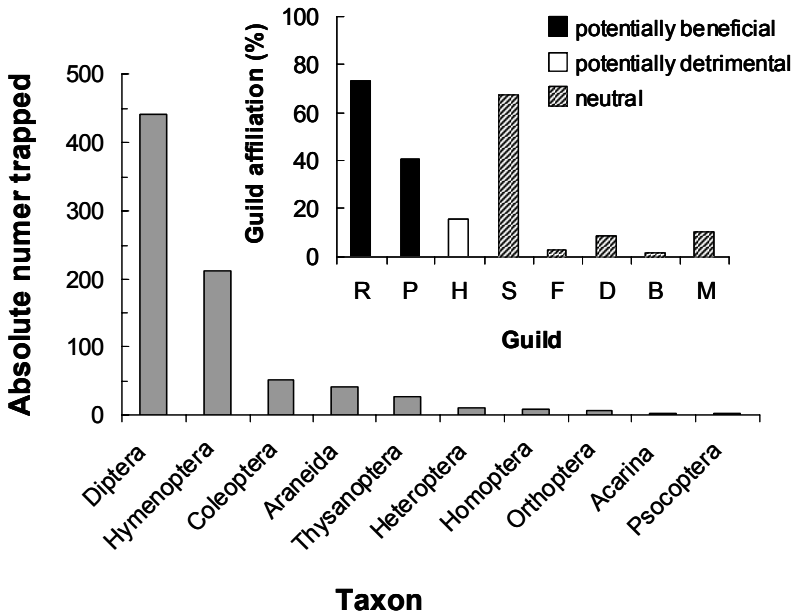


Figure 5. Arthropods taxa trapped on the sticky traps. Insert: Affiliation of trapped taxa to different guilds: **R**, predator/entomophaga; **P**, parasitoid; **S**, utilization of plant-derived resources including floral or extrafloral nectar, pollen and honeydew; **F**, frugivore; **H**, herbivore and flower feeder; **D**, detrivore including phytosaprophage and zoosaprophage; **B**, blood-sucking and ectoparasitic; **M**, fungivore. Arthropod groups were assigned to guilds according to nutritional or functional aspects whenever larval or adult stage feature the respective trait. Additionally, putative effects on the Lima bean are encoded in different colours of bars. Multiple affiliations per taxon were allowed. Sample size was two sticky traps per 14 tendril groups.

Table 2. Arthropod taxa trapped on the experimental tendrils with sticky traps.

Order	Taxon	Number (ind.)	Guild							
			R	P	S	F	H	D	B	M
Diptera	Dolichopodidae	150	l/a		a					
	Phoridae	103	l	a	a					
	Chloropidae	31	l	a	a		l			
	Tachinidae	24	l	a	a					
	Scatopsidae	22			a			l/a		
	Sciaridae	12					l	l/a		l/a
	Cecidiomyidae	12	l	a	a		l			l/a
	Psychodidae	11			a			l		
	Drosophilidae	10				l/a				l/a
	Culicidae	9	l		a		l	l	a	
	Canacidae	7		?						
	Platystomatidae	5			a		l	l		
	Asilidae	4	l/a							
	Asteiidae	3	l/a							
	Micropezidae	3			a			l/a		
	Tipulidae	3			a			l		
	Agromyzidae	2			a		l/a			
	Scenopinidae	2	l		a			l		
	Odiniidae	2								l
	Others	22								
Hymenoptera	Chalcidoidea	136	l/a	a	a					
	Formicidae	25	l/a		a					
	Braconidae	12	l/a	a	a					
	Sphecidae	7	l/a	a	a					
	Bethylidae	7	l/a	a	a					
	Pompilidae	6	l	a	a					
	Vespoidea	4	l/a	a	a	a				
	Ichneumonidae	3	l/a	a	a					
	Dryinidae	3	l/a	a	a					
	Cephidae	2			a		l/a			

continued →

Table 2. continued

Order	Taxon	Number (ind.)	Guild							
			R	**P**	**S**	**F**	**H**	**D**	**B**	**M**
	Tiphiidae	2	l/a	a	a					
	Others	3								
Coleoptera	Lathridiidae	10								l/a
	Coccinellidae	10	l/a				l/a			l/a
	Chrysomelidae	9					l/a			
	Staphylinidae	4	l/a	a	a	l	l/a			
	Curculionidae	4					l/a			
	Cucujidae	3	l/a					l/a		l
	Carabidae	2	l/a	a			l/a			
	Tenebrionidae	2	l/a		a	l/a	l/a	l/a		l/a
	Cleroidea	2								l/a
	Others	101								
Araneida		41	l/a							
Thysanoptera		26	l/a				l/a			a
Heteroptera	Tingidae	4					l/a			
	Hebridae	2	l/a							
	Others	2								
Orthoptera	Gryllidae	2	l/a				l/a			
	Others	4								
Acarina		3	l/a				l/a		l/a	
Psocoptera		2	l/a					l/a		l/a

Numbers of wasps and flies refer to the total number of insects trapped. 'Others' comprise individuals that either could only be determined to the order level or taxa of which just one individual was trapped. The observed arthropod groups were assigned to the following guilds according to nutritional or functional aspects: **R**, predator/entomophaga; **P**, parasitoid; **S**, utilization of plant-derived resources including floral or extrafloral nectar, pollen and honeydew; **F**, frugivore; **H**, herbivore and flower feeder; **D**, detrivore including phytosaprophage and zoosaprophage; **B**, blood-sucking and ectoparasitic; and **M,** fungivore. The affiliation to a guild is further differentiated by the developmental stage of each taxon (**l** = larval, **a** = adult) that features the respective trait.

may be classified as 'tourists' of the Lima bean and are likely to have a neutral effect on the plant (Table 2, Fig. 5).

A closer look at the arthropod taxa trapped revealed that Dolichopodidae (34 % of all trapped Diptera), Phoridae (24 %) and Chloropidae (7 %) were the most abundantly trapped Dipterans (Table 2). All three groups share parasitoid and predacious life habits and are known to occasionally feed on EFN (Table 2). Members of the Chalcidoidea contributed preponderance to the captured Hymenoptera (64 %). The individuals trapped of this superfamily of parasitoid wasps belonged to 16 different families with Eulopidae (13 % of all trapped Hymenoptera), Encyrtidae (12 %) and Pteromalidae (8 %) being most frequently trapped. Among the hymenopterans, Formicidae (12 %) and Braconidae (6 %) were the most often captured non-chalcid families.

Discussion

Treatment effects on the plant

The aim of the present study was to experimentally separate the protective effect of the two indirect defences EFN secretion and VOC emission in Lima bean under field conditions. The performance of plants, which have been induced by spraying with JA and thus had increased amounts of VOCs and EFN was monitored, and compared to plants where the amount of either EFN or VOCs was artificially increased. Interestingly, it turned out in the course of the experiment that a clear separation of these two defences was not possible: VOCs induced the secretion of EFN (Fig. 2). Hence, the tendrils of the volatile group have experienced the combined defensive effect of both VOCs and EFN to an extent, comparable to the tendrils of the JA group. Obviously, these two defences are not only connected by the shared signalling molecule JA (HEIL, 2004a), but also airborne VOCs are implicated in the induction of EFN secretion within one or between two conspecific plant individuals.

A quantitative comparison of the five experimental groups with regard to the development of fitness-relevant plant parameters revealed that the tendrils of the JA, the volatile and the EFN group had benefited of the respective treatment. These tendrils had suffered of less herbivory by leaf-chewing herbivores and bore fewer dead shoot tips than the two control groups. The picture, however, changes when also the other measured fitness-relevant plant parameters are included. For the number of living shoot tips, leaves, and inflorescences only the tendrils treated with VOCs and EFN differed significantly from the controls (Fig. 3). A possible explanation for the weaker defensive effect experienced by the JA-treated tendrils could be that induction with JA incurred allocation costs to the treated tendrils, which were greater then costs

incurred by the VOC-induced EFN secretion (HEIL & BALDWIN, 2002; STRAUSS *et al.*, 2002). Beyond stress-related reactions such as insect and disease resistance, JA is well known to be also involved in various physiological or morphological changes which are not necessarily related to resistance (CREELMAN & MULLET, 1997). Given that these are costly in terms of metabolic resources, the induction of such processes may have affected the measured fitness parameters and thus could explain the weaker defensive status of JA-treated tendrils.

The development of the fitness-relevant plant parameters measured in the JA-treated group and the two controls strongly resembled those of a preceding study (HEIL, 2004a). In both studies, JA-treatment led to an increase in the number of newly produced leaves and a decrease of both the number of dead shoot tips as well as the herbivory rate. The induction of these two indirect defences has benefited Lima bean plants in two independent studies performed in two consecutive years, underlining the importance of EFN secretion and VOC emission for Lima bean defence in nature.

JA-application could have additionally induced putative direct defences (HALITSCHKE & BALDWIN, 2004). However, no such alternative defence strategy has been described for Lima bean until now. In its close relative *Phaseolus vulgaris*, ENGLISH LOEB and KARBAN (1991) did not find evidence for an induced direct resistance to spider mites. However, a protective effect of a direct defence, which in our long-term experiment could have been induced after JA-application, cannot be excluded. In this case, the direct defence did not significantly contribute to plant protection, because tendrils with increased amounts of EFN only (EFN group) or EFN and volatiles combined (volatile group) performed better than the tendrils with EFN, volatiles and the putative direct defence (JA group) (Fig. 3).

EFN and volatile treatment were adjusted to resemble strongly induced Lima bean tendrils. The qualitative and quantitative composition of the emitted VOC blend and the secreted EFN within 24 h, was determined in preceding experiments (HEIL, 2004a; KOST & HEIL, 2005a, b). Due to the sample size required to achieve sufficient statistical power, the minimal interval between treatment rounds was 3 to 4 days. Thus, the amount of both artificial VOC and EFN blend applied was scaled up to cover the mean amounts naturally produced by a plant within this time period. Therefore, our mode of application did likely not meet the natural situation. Nevertheless, the amount of VOCs and EFN applied artificially ranged largely below the physiological expression limit of these two indirect defences (HEIL, 2004a; KOST & HEIL, 2005a, b).

Treatment effects on the insect community

Ants were by far the most dominant insect group observed on experimental tendrils and were significantly more attracted to the tendrils of the JA, volatile and EFN group (Fig. 4). Attraction of ants to EFN is a well known defensive mechanism (BENTLEY, 1977a; BUCKLEY, 1982) that has been reported to translate into an enhanced plant protection in some studies (BENTLEY, 1977b; HEIL *et al.*, 2001; LABEYRIE *et al.*, 2001; OLIVEIRA *et al.*, 1999), yet not in others (BECERRA & VENABLE, 1989; BOECKLEN, 1984; O'DOWD & CATCHPOLE, 1983; RASHBROOK *et al.*, 1992). Although chemical cues are important for ants, since they are frequently involved in home range-marking or mediating social interactions (KEELING *et al.*, 2004; VANDER MEER *et al.*, 1998), relatively little is known on whether also plant-derived volatiles can influence ant behaviour. Some reports are available on the role of yet unidentified plant chemicals for the orientation of ants to their host plant (AGRAWAL & DUBIN-THALER, 1999; DJIETO-LORDON & DEJEAN, 1999a, b; FIALA & MASCHWITZ, 1990) or the patrolling behaviour within a host plant (BROUAT *et al.*, 2000). In Lima bean, herbivory induces both VOC emission and EFN secretion (HEIL, 2004a). Ants could use the herbivore-induced volatiles as long-distance cues to detect patches of increased availability of EFN which at the same time are characterized by an increased presence of herbivores and hence potential prey. However, preliminary experiments with *Camponotus novogranadensis*, one of the three most dominant ant species visiting Lima bean at the two study sites (KOST & HEIL, 2005a), indicated that given the choice in a Y-olfactometer (HEIL, 2004b) between lanolin paste and lanolin paste containing volatiles, these ants did not preferentially choose the olfactometer arm with volatiles, although they significantly chose an arm with mashed banana in previous trials (C. KOST, unpublished data). More experiments are needed to study the role of plant volatiles for ant foraging behaviour.

Also wasps were significantly more attracted to the tendrils of the JA, volatile and EFN group, yet in much smaller numbers than ants. The majority of the wasps trapped on the experimental tendrils were characterized by predacious or parasitoid life habits (Table 2), supporting a previous study on sticky-trap captures of untreated and EFN treated Lima bean tendrils (KOST & HEIL, 2005a). These observations suggest that not only ants, but also wasps have contributed to the protection of the treated tendrils. Unfortunately it remains unclear which indirect defence - volatiles or EFN - was mainly responsible for wasp attraction. Wasps feeding on EFN has been reported for several different plant species (see KOPTUR, 1992 for review), yet so far only one study demonstrated that this mechanism may translate

into a fitness-benefit for the EFN-secreting plant (CUAUTLE & RICO-GRAY, 2003).

Comprehensive evidence for volatile-mediated wasp attraction is available from laboratory-based studies (e.g. DU *et al.*, 1996; TAKABAYASHI *et al.*, 1995; TURLINGS *et al.*, 1990), yet relatively few addressed this issue in the field. Among them, JAMES (2005) identified methyl salicylate as attractant for parasitic families such as Encyrtidae and Mymaridae. Methyl salicylate was also constituent of the artificial volatile blend used in this study and these families were also trapped on our experimental tendrils. The same holds true for Braconidae. These parasitic wasps were attracted to (*Z*)-3-hex-3-enyl acetate and (*Z*)-jasmone (JAMES, 2005), two further constituents of our artificial VOC blend. A more detailed analysis of the attractive effects of EFN and VOCs on wasps is required, in which especially single blend constituents should be field-tested.

Flies responded differently to tendril treatment than ants and wasps, since they were only significantly attracted to the tendrils of the nectar group (Fig. 4). This observation suggests that flies were more attracted to the artificial nectar than to airborne volatiles. The community of trapped Diptera covered a very diverse spectrum of feeding habits ranging from predacious or parasitoid taxa over herbivorous taxa to taxa feeding on detritus or fungi (Table 2). This heterogeneous composition complicates a clear functional assignment of the trapped flies. In most of the cases it appears likely that the trapped flies simply exploited the offered EFN as an additional food source rather than contributing to a larger extent to plant protection. In this case, EFN consumption without providing the plant with any mutual benefit such as plant protection could cause important ecological costs, because the EFN-producing plant would be less protected against herbivores (HEIL, 2002; HEIL *et al.*, 2004a). However, this is speculation and needs to be confirmed in future experiments.

Synopsis

Ecological studies on the benefit of indirect defences generally focussed on the protective effect of either one single defensive trait or the combined effect of several defences such as after simultaneous induction with elicitors like JA. While this simplification is easy to understand from the viewpoint of experimental feasibility, such univariate approaches may be inappropriate since they do not appreciate the complex interplay of several plant defences within one plant species (DUFFEY & STOUT, 1996). This study takes a first step into this direction.

The mere application of artificial EFN (nectar group) resulted in a fitness benefit always stronger or quantitatively similar to the one experienced by the tendrils of the JA- and the VOC-treatment (Fig. 3).

Moreover, ants observed on the experimental tendrils showed an overwhelming numerical superiority over all other arthropod groups (Fig. 4). These two observations suggest that under our experimental conditions the secretion of EFN was more important for plant defence than the VOC-mediated arthropod attraction.

However, our experimental design does not allow excluding an attractive effect of airborne VOCs on flying or crawling arthropods. This issue needs to be addressed in future studies, which should especially focus on the role of volatiles and EFN for the short- and long-distance attraction of herbivores and plant defenders. Several of the arthropod taxa which have been identified in this study could serve as possible targets for such analyses. Furthermore, laboratory- and field-based experimentation is needed to study whether inductive situations exist in which either the volatile emission or the EFN secretion is differentially up- or down-regulated or if both defences always respond similarly to herbivore attack.

Acknowledgements

We thank W. BOLAND for the possibility to use all the facilities of the Department of Bioorganic Chemistry and S. ADOLPH and M. MEKEM SONWA for technical advice on organic synthesis. Financial support by the German Research Foundation (DFG Grant He 3169/2-1, 2) and the Max-Planck-Society is gratefully acknowledged.

Manuscript V

Dehydrogenation of ocimene by active carbon: Artefact formation during headspace sampling from leaves of *Phaseolus lunatus*

Mesmin Mekem Sonwa[1], Christian Kost[1], Anja Biedermann[1], Robert Wegener[2], Stefan Schulz[2] and Wilhelm Boland[1]*

Submitted to *Tetrahedron*

[1] Department of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Beutenberg Campus
Hans-Knöll-Str. 8
D-07745 Jena, Germany

[2] Department of Organic Chemistry
Technical University of Braunschweig
Hagenring 30
D-38106 Braunschweig, Germany

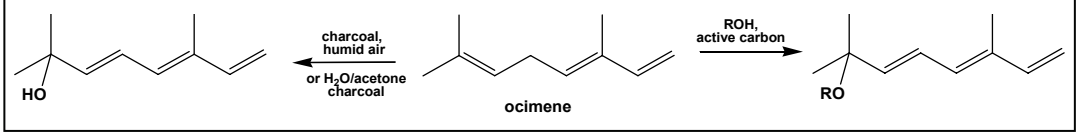
*** Corresponding author:**
Wilhelm Boland
Department of Bioorganic Chemistry
Max Planck Institute for Chemical Ecology
Beutenberg Campus, Hans-Knöll-Str. 8
D-07745 Jena, Germany
Phone: + + 49 - 3641 - 57 12 00
Fax: + + 49 - 3641 - 57 12 02
E-mail: boland@ice.mpg.de

Graphical Abstract

Dehydrogenation of ocimene by active carbon: Artefact formation during headspace sampling from leaves of *Phaseolus lunatus*

Mesmin Mekem Sonwa[1], Christian Kost[1], Anja Biedermann[1], Robert Wegener[2], Stefan Schulz[2], and Wilhelm Boland[1*]

[1] *Max-Planck-Institut für Chemische Ökologie, Hans-Knöll-Str. 8, D-07745 Jena, Germany*
[2] *Technical University of Braunschweig, Department of Organic Chemistry, Hagenring 30, D-38106 Braunschweig, Germany*



Abstract

The blend of volatiles emitted from jasmonate-treated Lima bean (*Phaseolus lunatus*) leaves comprised two ocimene-derived artefacts that were only present, if the compounds were collected on active carbon traps. Identified were (3*E*,5*E*)-2,6-dimethyl-3,5,7-octatrien-2-ol (**6**) and (3*E*,5*E*)-2,6-dimethyl-1,3,5,7-octatetraene (**5**) resulting from oxidation of ocimene (**2**) by active carbon in presence of humid air. The catalytical capacity of the active carbon could be exploited for a rapid and efficient functionalization of ocimene to give the (3*E*,5*E*)-2-alkoxy-3,5,7-octatrienes (**14**), (**15**), and (**16**) in methanol, ethanol, or isopropanol as solvents.

Keywords: *Phaseolus lunatus*, charcoal, induced volatiles, ocimene, closed-loop-stripping analysis (CLSA), SPME

1. Introduction

The Lima bean (*Phaseolus lunatus*) exhibits selective response patterns to different herbivores (OZAWA *et al.*, 2000) or chemical elicitors like jasmonic acid (KOCH *et al.*, 1999). The different blends of volatiles which are emitted upon herbivore feeding are generally believed to attract natural enemies of the herbivores and thereby function as an indirect plant defence (DE MORAES *et al.*, 1998; GOUINGUENE *et al.*, 2003). Thus, precise knowledge on the qualitative and quantitative composition of the volatile blends is essential for understanding multitrophic interactions. Analysing the volatile organic compounds emitted by the Lima bean after treatment with jasmonic acid (JA), we observed two rare monoterpenes that were only present in the volatile blend collected with the closed-loop-stripping method (CLSA) (DONATH & BOLAND, 1995) (Tab. 1), yet were absent if volatiles were collected on polydimethyl-siloxane-coated fibres with solid-phase-microextraction (SPME) (ARTHUR & PAWLISZYN, 1990). Trapping ocimene with commercial charcoal traps and subsequent analysis of the desorbed compounds with GC-MS again revealed the presence of **5** and **6**, whereas simultaneous trapping of the same headspace with SPME did not result in the detection of these compounds.

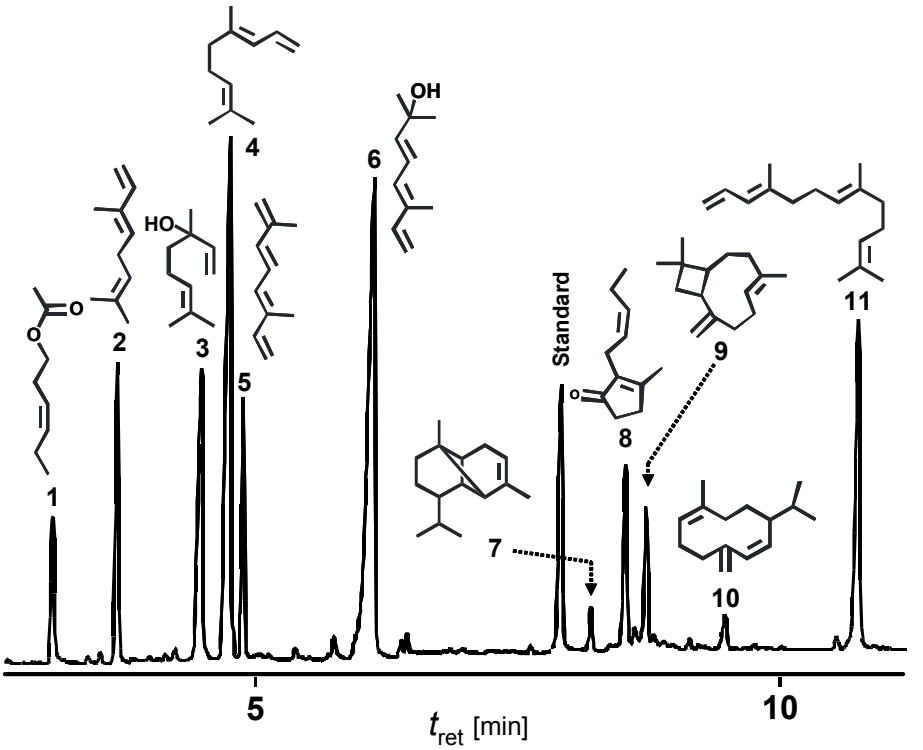


Figure 1. Gas chromatographic separation of volatiles collected from the gas phase around Lima bean leaves treated with jasmonic acid. Compounds were absorbed on charcoal traps (DONATH & BOLAND, 1995) and analyzed by GLC-MS after desorbtion with CH_2Cl_2.

The two substances **5** and **6** have been previously identified in the headspace of hyacinth flowers where they could only be detected in headspace samples yet not in extracts or distillates of the same plant material (KAISER & LAMPARSKY, 1977). Furthermore, the substances could be clearly identified as artefacts derived from (*E*)-β-ocimene (**2**), which is formed in the presence of active charcoal (BRUNKE *et al.*, 1993). Here we report on this carbon-mediated reaction that rapidly and stereoselectively leads to a functionalisation of 1,3-dienes.

Table 1. Amount of **2**, **5** and **6** released by three Lima bean plants 24 h after spraying with jasmonic acid. Area has been referenced to plant dry weight (DW).

	Mean area ± SD (count seconds 10^8 *g DW^{-1})		
Sampling method	**2**	**5**	**6**
Charcoal trap	24 ± 15	2.9 ± 1	6.5 ± 5
SPME	2.0 ± 1	-	-
Charcoal trap[a]	34.2	0.4	4.7

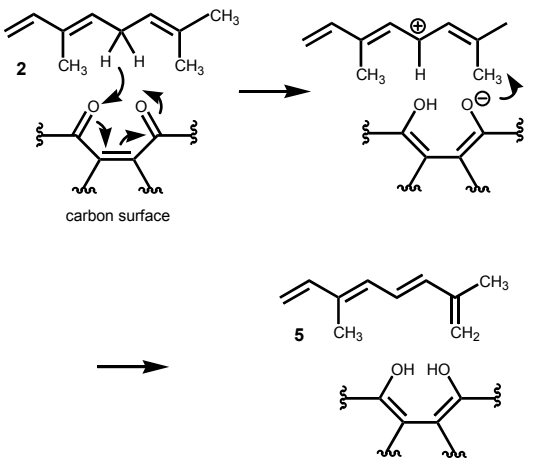
[a] eluate re-trapped with SPME, - not detected

2. Results and discussion

2.1. Active carbon catalysed oxidation of ocimene.

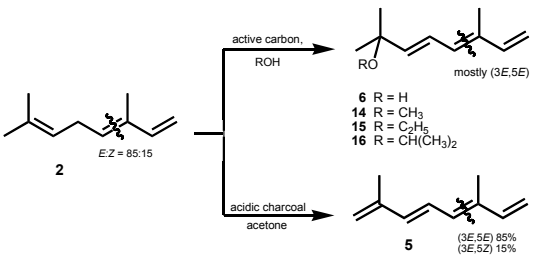
Active carbon is a well known support for noble metals, such as palladium and platinum (RODRIGUEZ-REINOSO, 1998). COUGHLIN *et al.* (1969) have reported redox reactions catalysed by carbon, halogenation and even polymerization. Active carbon is also known to catalyse the oxidation of fluorene as well as halogen exchange reactions (YANG & JOHNSON, 1977). MAKINO *et al.* (1992) demonstrated that activated active carbon catalyses the conversion of physalin B into 25-hydroxyphysalin B under very mild conditions. Although the mode of action of active carbon has not yet been established, carbon-oxygen surface complexes have been postulated. Other reactions may be due to the acidic or alkaline nature of the carbon surface (YANG & JOHNSON, 1977). TOMITA *et al.* (1971) postulated free radicals as carbon surface active sites catalysing the reaction.

To clarify whether radicals play a role in the active carbon catalysed hydroxylation of ocimene, active carbon was either treated with the radical starter AIBN or with the radical scavenger BHT prior to the addition of ocimene. In both cases, no change was found in the catalytic activity of active carbon, suggesting that radicals are not involved in the oxidation process. Alternatively, oxygen-functionalized 1,4-quinonoid centres on the surface of active carbon have been recently discussed for the dehydrogenation of ethylbenzene to styrene (ZACHARIA, 2004; ZHU *et al.*, 2002). Accordingly, the initial step of the dehydrogenation of ocimene is the transfer of a hydride to a surface carbonyl (Scheme 1).



Scheme 1. Postulated mechanism of the dehydrogenation of ocimene by 1,4-quinonoid centres on the surface of active carbon; modified after ZACHARIA (2004). The proposed mechanism resembles dehydrogenation reactions mediated by DDQ (BRAUDE *et al.*, 1960).

Subsequent loss of a proton from the resonance-stabilized cationic intermediate to the resulting phenolate anion completes the sequence and yields the tetraene **5**. If a nucleophile is present, this could compete with the elimination and lead to functionalized octatrienes as shown in Scheme 2. The observation that the same dehydrogenation could be also achieved by DDQ (BRAUDE *et al.*, 1960) by simply stirring of 1 eq. of ocimene in tetrachloromethane with 3 eq. of DDQ at r. t. suggests that the transformation may indeed follow the mechanistic model of Scheme 1. To verify this reaction course, moistened active carbon was stirred in hexane and oxygen was removed by passing argon through the suspension (1 h) followed by addition of ocimene (**2**). After 72 h, GLC-MS analysis revealed, besides unreacted ocimene (30 %), the presence of the alcohol **6** (35 %) along with the tetraene **5** (30 %), demonstrating that the presence of active carbon is sufficient to achieve the reaction. In a more polar medium (acetone/water, access of air), the oxidation proceeded faster, and after 48 h most of **2** (60 %) was converted to the alcohol **6** and the hydrocarbon **5**. Control experiments without active carbon did not result in oxidation of ocimene. Since the cationic intermediate of Scheme 1 principally allows a reaction with nucleophiles, we tested several alcohols as solvents and reactants (Scheme 2).



Scheme 2. Oxidative transformations of ocimene with active carbon.

In fact, simple stirring of ocimene with active carbon in methanol generated 2-methoxy-2,6-dimethyl-octa-3,5,7-triene (**14**), known as a minor constituent of the essential oil of *Narcissus geranium* (VAN DORT *et al.*, 1993). Previous syntheses of **14** required lengthy multi-step procedures (VAN DORT *et al.*, 1993). The starting mixture of (3*E,Z*)-ocimene showed a faster conversion of the (3*E*)-isomer resulting in oxidation products with higher configu-

rational purity than the educts. According to NMR, the new double bond was exclusively *trans*. A similar reaction was performed in ethanol and yielded (3*Z*,5*E*)-7-ethoxy-3,7-dimethylocta-1,3,5-triene (**15**) with a non-optimized yield of 13 % after 48 h and 18 % after 72 h. In isopropanol (3*Z*,5*E*)-2-isopropxy-2,6-dimethyl-octa-3,5-diene (**16**) was obtained albeit in low yield (5 % after 48 h and 6 % after 72 h). In *t*-butanol only trace amounts of the corresponding *t*-butylether were detected (ca. 1 %), demonstrating that only primary and secondary alcohols can be used.

If the acidic character of the active carbon was increased by pre-treatment with nitric acid prior to use (acetone as solvent), ocimene was directly transformed to the hydrocarbon **5**. Scope and limitations of this novel and operationally simple, active carbon-mediated, functionalisation of 1,3-dienes are currently investigated.

3. Experimental

3.1. General information

^{1}H and ^{13}C NMR: Bruker Avance 400 spectrometer (Bruker, D-76287 Rheinstetten, Karlsruhe, Germany). Chemical shifts of ^{1}H and ^{13}C NMR are given in ppm (δ) based on solvent peaks: $DCCl_3$ 7.26 ppm (^{1}H NMR) and 77.70 ppm (^{13}C NMR); CD_3OD 3.31 ppm (^{1}H NMR) and 49.00 ppm (^{13}C NMR). GC-MS spectra were recorded on a Finnigan GCQ, equipped with an Alltech DB5 (0.25 mm x 30 m) column. Helium at 30 cm min^{-1} served as carrier gas. Compounds were eluted under programmed conditions starting from 40 °C (4 min) and then at 10 °C min^{-1} to 200 °C followed 55 °C min^{-1} to 280 °C (3 min). Activated carbon was purchased from Aldrich (Cat.: 16,155-1, 82024 Taufkirchen, Germany).

3.2. Plant material

Experiments using jasmonic acid-treatments were performed with Lima bean plants (*Phaseolus lunatus* var. Jackson Wonder Bush). Seeds were obtained from Kelloggs Seed Inc., USA. Individual plants were grown from seeds in plastic pots (Ø = 5.5 cm) with sterilized potting soil at 21 - 23 °C and 50 - 60 % humidity using daylight fluorescent tubes at approximately 470 µE $m^{-2}s^{-1}$ with a photophase of 14 hours.

3.3. Induced biosynthesis of volatiles and collection of emitted compounds

10- to 14-d-old plantlets of *P. lunatus* with two fully developed primary leaves were cut with razor blades and immediately placed into glass vials containing an aqueous solution of jasmonic acid (4 ml of a 1 mmol aqueous solution of jasmonic acid). The vials with the plants were transferred to 5 l desiccators and the emitted volatiles collected over a period of 48 h with the closed loop stripping method (CLSA; DONATH & BOLAND, 1995)

using active carbon traps (1 mg of charcoal, CLSA-Filter, Le Ruisseau de Montbrun, F-09550 Daumazan sur Arize, France). At the end of the collection period, the trapped volatiles were eluted from the active carbon by washing three times with 10 µl of dichloromethane. 1-Bromodecane was used as an internal standard (400 µg ml^{-1}). The collected samples were analysed by GLC-MS and indicated, besides other compounds shown in Figure 1, the presence of **5** and **6**. Alternatively, volatiles were collected with SPME fibres coated with carboxen™-polydimethyl-siloxane (Supelco/Aldrich, Cat.: 57318, D-82024 Taufkirchen, Germany) (ARTHUR & PAWLISZYN, 1990). Subsequent thermal desorbtion from the fibre and GLC-MS analysis confirmed the presence of all compounds shown in Figure 1 except of the ocimene-derived artefacts **5** and **6**. The observation that re-trapping an eluate of an active carbon trap measurement with SPME and analysing it with GLC-MS, lead to the compounds **5** and **6** suggested that both compounds originated from the first collection process using active carbon traps.

*3.4. Active carbon catalyzed hydroxylation of ocimene to 2,6-dimethylocta-3,5,7-triene-2-ol (**6**)*

30 mg (0.22 mmol) ocimene (mixture of 3*E,Z*-isomers, ca. 85:15) was added to a suspension of active carbon (0.5 g) in 31 ml acetone/water (30:1, v:v) and the mixture was stirred at room temperature. After 48 hours, GC-MS analysis showed that 60 % of ocimene (largely the (3*E*)-isomer) was transformed. After 72 hours, the (3*E*)-isomer and ca. 50 % of the (3*Z*)-isomer were hydroxylated. After 96 h both isomers were completely transformed. Following addition of water (20 ml) the product was extracted with pentane (3 x 20 ml), concentrated *in vacuo*, and purified by chromatography on silica gel. Yield: 14 mg (41 %). The spectroscopic data were in agreement with literature data (BRAUDE *et al.*, 1960).

3.5. Functionalisation of ocimene, general procedure

Activated carbon (2.5 g) was suspended with stirring in either methanol (120 ml), ethanol or isopropanol (415 ml) followed by addition of ocimene (198 mg, mixture of 3*E*/*Z* isomers, ca. 85:15). After stirring at r.t. for 48 h or 72 h, the reactions were stopped and the activated carbon was removed by filtration. GC-MS analysis demonstrated the formation of 2-methoxy-2,6-dimethylocta-3,5,7-triene (**14**) from methanol, 2-ethoxy-2,6-dimethylocta-3,5,7-triene (**15**) from ethanol, and 2-isopropoxy-2,6-dimethylocta-3,5,7-triene (**16**) from isopropanol as solvent and nucleophile. For isolation of the ethers the solvents were removed under reduced pressure and the products purified by column chromatography. Owing to the high volatility of the ethers, removal of the rather

large amounts of solvents resulted in low yields of isolated products.

3.6. Dehydrogenation of ocimene with acidic active carbon

100 ml of 66 % nitric acid was added to 20 g of activated carbon and the mixture was stirred for 18 hours. Nitric acid was removed and the active carbon was washed with distilled water until the pH of the suspension of active carbon in water stabilized at 5.5. Water was removed and the active carbon was dried at 80 °C for 16 hours. 20 mg of the oxidized active carbon was used for the transformation of ocimene, following the same protocol as for untreated active carbon described in 3.7. After 24 hours, GC-MS analysis revealed complete conversion of ocimene to the hydrocarbon **5**.

3.7. Spectroscopic data

*3.7.1. (3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene (**5**): (3E,5E)-(**5**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.55 (dd, 1H, $J_{4,5}$ = 15.4 Hz, $J_{4,5}$ = 11.5 Hz, H-4), 6.45 (dd, 1H, $J_{7,8a}$ = 10.6 Hz, $J_{7,8b}$ = 17.4 Hz, H-7), 6.57 (d, 1H, H-5), 6.14 (d, 1H, H-5), 5.41 (d, 1H, H-8b), 5.04 (d, 1H, H-8a), 5.00 (s, 4H, H-1), 1.90 (d, 5H, CH_3), 1.89 (s, 5H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.9 (C-4), 141.5 (C-7), 136.5 (C-3), 136.3 (C-6), 131.9 (C-5), 125.7 (C-4), 117.1 (C-1), 112.7 (C-8), 18.6 (C6-CH_3), 12.2 (C2-CH_3); EIMS (m/z) 134 (63), 120 (10), 119 (100), 117 (21), 106 (9), 105 (23), 103 (11), 93 (14), 92 (20), 91 (91), 79 (24), 78 (11), 77 (33), 65 (15), 55 (11), 53 (9), 51 (9), 41 (15), 39 (15).

*3.7.2. (3E,5E)-2-Methoxy-2,6-dimethyl-3,5,7-octatriene (**14**)*

Yield: 117 mg (60 %). (3*E*,5*E*)-**14** (85 %) and (3*E*,5*Z*)-**14** (15 %) according to NMR. (3*E*,5*E*)-**14:** ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 6.41 (dd, 1H, $J_{4,5}$ = 11.18 Hz, $J_{4,3}$ = 15.59 Hz, H-4), 6.33 (dd, 1H, $J_{7,8a}$ = 17.24 Hz, $J_{7,8b}$ = 11.18 Hz, H-7), 6.01 (d, 1H, H-5), 5.66 (d, 1H, H-3), 5.15 (d, 1H, H-8a), 4.98 (d, 1H, H-8b), 3.10 (s, 3H, O-CH_3), 1.81 (s, 3H, C6-CH_3), 1.24 (s, 6H, C2-$(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 141.54 (C-3), 140.32 (7), 135.70 (C6), 131,18 (C5), 125.97 (C-4), 113.07 (C-8), 75.51 (C-2), 50.85 (O-CH_3), 26.31 (C2-$(CH_3)_2$), 12.45 (C6-CH_3) ; EIMS (m/z) 166 (68), 165 (11), 152 (7), 151 (82), 150 (15), 137 (13), 136 (12), 135 (35), 133 (26), 123 (18), 119 (100), 118 (14), 117 (24), 109 (10), 107 (35), 105 (33), 93 (46), 92 (22), 91 (79), 90 (21), 86 (25), 85 (11), 79 (28), 76 (54), 73 (17), 67 (6), 65 (11), 59 (64), 58 (25), 55 (13), 43 (46).

*3.7.3. (3Z/5E)-2-Ethoxy-2,6-dimethylocta-3,5,7-triene (**15**)*

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.49 (dd, 1H, $J_{7,8a}$ = 17.1 Hz, $J_{7,8b}$ = 11.0 Hz, H-7), 6.40 (ddd, 1H, $J_{3,4}$ = 17,3 Hz, H-7), 6.10 (d, 1H, $J_{4,5}$ = 10.4 Hz, H-5), 5.78 (d, 1H, $J_{3,4}$ = 15.4 Hz, H-3), 5.24 (d, 1H, $J_{7,8a}$ = 17.1 Hz, H-8a), 5.07 (d, 1H, $J_{7,8a}$ = 11 Hz, H-8b),

3.35 (q, 2H, OCH_2CH_3), 1.90 (s, 3H, CH_3), 1.34 (d, 6H, CH_3), 1.18 (t, 3H, $-CH_2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 141.8 (C-3), 140.8 (C-7), 135.1 (C-6), 130.9 (C-5), 125.6 (C-4), 112.5 (C-8), 74.9 (C-2), 58.0 (C-1'), 26.5 (2 x C-2 CH_3), 16.1 (C-6 CH_3), 12.0 (C-2'); EIMS (m/z) 180 ($M^{+\bullet}$, 54), 165 (66), 151 (19), 136 (51) 135 (52), 121 (31), 119 (72), 109 (61), 107 (68), 105 (40), 93 (100), 91 (95), 79 (41), 77 (60), 59 (44), 55 (34). EI-HRMS calcd. for $C_{12}H_{20}O$ 180.15142, found 180.15157.

*3.7.4. (3Z/5E)-2-Isopropxy-2,6-dimethyl-octa-3,5-diene (**16**)*

^1H NMR ($CDCl_3$, 400 MHz) δ 6.38 (dd, 1H, $J_{7,8a}$ = 17.1 Hz, $J_{7,8b}$ = 11.0 Hz, H-7), 6.34 (dd, 1H, $J_{3,4}$ = 17.3 Hz, H-4), 6.01 (d, 1H, $J_{4,5}$ = 11.0 Hz, H-5), 5.72 (d, 1H, $J_{3,4}$ = 15.4 Hz, H-3), 5.20 (d, 1H, $J_{7,8a}$ = 17.1 Hz, H-8a), 4.98 (d, 1H, $J_{7,8a}$ = 11 Hz, H-8b), 3.60 (sept., 1H, $OCH(CH_3)_2$), 1.71 (s, 3H, CH_3), 1.22 (d, 6H, CH_3), 1.18 (d, 6H, $-CH(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 141.2 (C-3), 140.3 (C-7), 135.1 (C-6), 131.0 (C-5), 124.8 (C-4), 112.6 (C-8), 75.4 (C-2), 64.8 (C-1'), 27.1 (2 x C-2 CH_3), 25.0 (2 x C-2'), 12.1 (C-6 CH_3); EI-MS (m/z) 194 ($M^{+\bullet}$, 16), 179 (6), 152 (13), 151 (15), 137 (25) 135 (24), 119 (22) 109 (100), 107 (31), 93 (56), 91 (46), 81 (27), 77 (30), 67 (14), 55 (12). EI-HRMS calcd. for $C_{13}H_{22}O$ 194.16707, found 194.16813.

3.7.5. Dehydrogenation of ocimene with DDQ

Ocimene (40 mg, 0.3 mmol, mixture of 3*E,*3*Z* isomers) was dissolved in carbon tetrachloride (15 ml) and DDQ (0.20 g, 0.9 mmol) was added with stirring. Stirring was continued for 48 h under argon at room temperature. After 48 h GC-MS revealed the exclusive formation of **5**. Owing to the absence of water no concomitant formation of the alcohol **6** was observed.

Acknowledgements

Rearing of plant and insect cultures by ANGELIKA BERG is gratefully acknowledged. We thank the Fonds der Chemischen Industrie, Frankfurt a. M., for financial support.

8. General discussion

The present thesis provides substantial evidence for the functioning of indirect defences in nature and their role for mediating intra- and interspecific interactions of the Lima bean (*Phaseolus lunatus*). To cope with its herbivorous enemies, Lima bean has developed two indirect defence strategies which are both inducible upon herbivore damage (Manuscript I). These are the secretion of extrafloral nectar (EFN) and the emission of volatile organic compounds (VOCs). Both of which attracted predacious and parasitoid arthropods that defended the plant against attacking herbivores and thus exerted a beneficial effect on the Lima bean (Manuscript II, III and IV). Besides their role as host-location cue for members of the third trophic level, VOCs are also implicated in signalling processes between herbivore-damaged and undamaged plants. VOCs induced by feeding herbivores had an inductive effect on the EFN secretion rate of neighbouring, conspecific plants or plant parts (Manuscript III and IV). Hence, they may serve as a signal for an impending herbivore attack which cause all bean tendrils positioned within a patch of increased herbivore pressure to precautionary activate an indirect defence.

The role of VOCs for plant defence

Price *et al.*'s (1980) seminal review on the "influence of plants on interactions between insect herbivores and natural enemies" generated a wealth of interesting studies demonstrating that members of the third trophic level can use plant-derived VOCs to locate their host or prey. Although the idea that plants and the natural enemies of their herbivores cooperate with the latter acting as bodyguards is intuitively appealing (STRONG & LARSSON, 1994), this hypothesis has to bear scrutiny of whether or not it fulfils the following assumptions (DICKE & VET, 1999; GODFRAY, 1995; JANSSEN *et al.*, 2002; VAN DER MEIJDEN & KLINKHAMER, 2000): i) the volatile signal should be sufficiently specific to allow natural enemies of the herbivore an effective localisation of their hosts/prey, ii) attraction of predators/ parasitoids should result in a reduced herbivore load that translates into an increased plant fitness, and iii) the benefit of volatile-attracted plant defenders should outweigh potential costs for the plant such as e.g. attraction of additional herbivores.

Evidence to support the first assumption in the *Phaseolus lunatus* system is mainly derived from laboratory studies. In Lima bean, the total amount of VOCs produced depends strongly on the quantity of herbivory inflicted (DE BOER *et al.*, 2004; HORIUCHI *et al.*, 2003a). The resulting bouquets were only attractive to predatory mites when being emitted from plants infested with median or high herbivore loads (HORIUCHI *et al.*, 2003a). Hence, foraging carnivores may assess food patch

quality via the quantity of VOCs emitted from attacked plants. Furthermore, testing the olfactory response of parasitoids and predators revealed their ability to discriminate between prey and non-prey herbivores on Lima bean (DE BOER *et al.*, 2004; DICKE & GROENEVELD, 1986; HORIUCHI *et al.*, 2003a; SABELIS & VAN DE BAAN, 1983), between Lima bean and other plant species attacked by the same herbivore species (DICKE *et al.*, 1990a; PETITT *et al.*, 1992) as well as between different plant cultivars of the Lima bean's close relative *Phaseolus vulgaris* attacked by the same herbivore species (DICKE *et al.*, 1990b). Although the ecological significance of these findings remains to be demonstrated under field conditions (HUNTER, 2002), they impressively illustrate the information content that is encoded in the plant-derived VOC plumes.

Until now it is unclear whether also the second assumption of a VOC-mediated top-down control is met in the *Phaseolus lunatus* system. Application of an artificial VOC blend to wild growing Lima bean plants attracted a large number of parasitoid and predacious insect species which presumably caused the reduced herbivory rate and the increased number of inflorescences as compared to controls without VOCs (Manuscript III and IV). However, VOCs also induced EFN. Therefore, the attractive effects of both VOCs and the VOC-induced EFN may have accounted for the beneficial effect experienced by the VOC-treated tendrils. A comparison of VOC-treated tendrils with tendrils where an artificial mixture of EFN was applied revealed similar effects of both treatments (Manuscript III and IV), indicating that the attractive effect of EFN seemed to be more important than the one of VOCs. In this case, VOCs would primarily function as an intra-individual and intra-specific signal rather than as an inter-specific signal directed towards the members of the third trophic level. This hypothesis needs to be addressed in further studies.

For other plant species such as *Nicotiana attenuata* in which a volatile-induced EFN secretion can be excluded due to the absence of extrafloral nectaries, VOCs attracted generalist predators which effectively decreased the plant's herbivore load (KESSLER & BALDWIN, 2001). Moreover, plants that were fed upon by parasitized lepidopteran larvae produced more seeds than plants attacked by unparasitized larvae (FRITSCHE HOBALLAH & TURLINGS, 2001; VAN LOON *et al.*, 2000). Thus it appears reasonable to assume that also the parasitoid and predatory species attracted to the Lima bean had similar effects on the plant.

The fitness benefit of the VOC-treatment to tendrils outweighed costs associated with the emission of volatile signals (Manuscript III and IV), and thus the third assumption was met. Potential costs of VOC emission that could have

Table 1. Plant species which share constituents of their herbivore-induced volatile blend with the Lima bean (*Phaseolus lunatus*).

Plant species	(Z)-3-hex-3-enyl acetate	(*E*)- β-ocimene	(*R*)-(-)-linalool	DMNT	methyl salicylate	(Z)-jasmone	β-caryophyllene	TMTT	Reference
Glycine max[1]					X				VAN DEN BOOM *et al.*, 2004
Laburnum anagyroides[1]		X			X		X		VAN DEN BOOM *et al.*, 2004
Lotus japonicus[1]	X	X		X	X				OZAWA *et al.*, 2000b
Medicago truncatula[1]	X			X	X		X	X	LEITNER *et al.*, 2005
Phaseolus vulgaris[1]	X		X	X			X	X	COLAZZA *et al.*, 2004
Robinia pseudo-acacia[1]		X	X		X				VAN DEN BOOM *et al.*, 2004
Vicia faba[1]	X		X				X	X	COLAZZA *et al.*, 2004
Vigna unguiculata[1]				X	X			X	VAN DEN BOOM *et al.*, 2004
Capsicum annuum[2]		X	X	X	X			X	VAN DEN BOOM *et al.*, 2004
Datura stramonium[2]					X		X	X	VAN DEN BOOM *et al.*, 2004
Nicotiana attenuata[2]	X	X	X		X				KESSLER & BALDWIN, 2001
Nicotiana tabacum[2]		X	X		X	X	X	X	VAN DEN BOOM *et al.*, 2004
Solanum nigrum[2]	X						X		SCHMIDT *et al.*, 2004
Solanum melalonga[2]		X		X				X	VAN DEN BOOM *et al.*, 2004
Solanum tuberosum[2]	ni.	ni.	ni.	ni.	ni.	ni.	X	ni.	WEISSBECKER *et al.*, 2000
Zea mays[3]	X		X	X	X		X	X	TAKABAYASHI *et al.*, 1995
Cucumis sativus[4]	X	X	X	X				X	TAKABAYASHI *et al.*, 1994b
Arabidopis thaliana[5]	X				X			X	VAN POECKE *et al.*, 2001
Brassica olearacea[5]	X								MATTIACCI *et al.*, 1995
Gossypium hirsutum[6]	X	X	X	X		X	X	X	RÖSE & TUMLINSON, 2004
Pinus sylvestris[7]		X							MUMM *et al.*, 2003
Humulus lupulus[8]					X		X	X	VAN DEN BOOM *et al.*, 2004
Ginkgo biloba[9]			X				X		VAN DEN BOOM *et al.*, 2004
Vitis vinifera[10]		X		X	X		X	X	VAN DEN BOOM *et al.*, 2004
Adenostyles alliariae[11]	X		X						KALBERER *et al.*, 2001
Petasites paradoxus[11]									KALBERER *et al.*, 2001

Plant families: [1] Fabaceae, [2] Solanaceae, [3] Poaceae, [4] Cucurbitaceae, [5] Brassicaceae, [6] Malvaceae, [7] Pinaceae, [8] Moraceae, [9] Ginkgoaceae, [10] Vitaceae, [11] Asteraceae; **X** emission compound significantly increased upon herbivore damage; **ni** not investigated.

occurred under our experimental conditions are the attraction of herbivorous arthropods to the applied VOCs (DICKE & VET, 1999; HILKER & MEINERS, 2002). Indeed, curculionid beetles, which are amongst the most important leaf-chewing herbivores of Lima bean in the study area, are attracted to VOCs of induced plants (HEIL, 2004b). Furthermore, VOCs could have attracted members of the fourth trophic level such as e.g. hyperparasites that would have had detrimental effects on the third trophic level (Manuscript I). However, the net effect for the plant was positive indicating that these costs were outweighed by the benefits of the applied VOCs.

The role of VOCs for plant-plant communication

The presented results provide several innovations in the field of plant-plant communication (Manuscript III): First, they demonstrate plant-plant communication for Lima bean. Second, they identify (*Z*)-3-hex-3-enyl acetate (possibly amongst others) as an airborne signal responsible for the observed defence reaction. Third, they are the first demonstration that an indirect defence mechanism is induced in the receiver plant, and fourth they provide field evidence that the observed mechanism may benefit *Phaseolus lunatus* under natural growing conditions. Lima bean plants obviously can use volatile signals from their environment to assess the risk of future herbivory in order to tailor their responses accordingly.

Theory predicts that plants will only respond to information released by damaged neighbours if the information is sufficiently reliable (ADLER & KARBAN, 1994; GETTY, 1996; KARBAN *et al.*, 1999). As discussed previously, the amount of VOCs emitted by a plant reflects the intensity of herbivore attack as well as the quantitative and qualitative composition of the VOC plume encodes the species of both the emitting plant and the attacking herbivore (Manuscript I). Interestingly, the compound which caused the strongest increase in the EFN secretion rate in downwind tendrils is known to be produced by many different plant species upon herbivore attack or mechanical damage: (*Z*)-3-hex-3-enyl acetate (Manuscript III, Tab. 1). Consequently, this compound is not only emitted from conspecifics, but also from other plant species that provide the Lima bean with information on the risk of future attack.

Generally, inducible defences suffer an unavoidable drawback, namely the lag time, which is needed to induce the defence. During this time the plant remains susceptible to herbivores and is likely to suffer greater tissue loss than a plant that has its defences already activated (JÄREMO *et al.*, 1999). VOC-mediated plant-plant communication may be an adaption to cope with this drawback, because yet undamaged individuals activate

their defences before they actually encounter an herbivore. (*Z*)-3-hex-3-enyl acetate in particular is known to be emitted rapidly after mechanical damage (ARIMURA *et al.*, 2000a) and thus allow the Lima bean to quickly responds to a current threat.

Evolutionary considerations immediately pose the following question: What is the benefit for the sender of this signal? At its natural growing site, Lima bean forms gregarious patches where it occurs closely entwined with conspecific individuals, other vines and the supporting vegetation. Many contact points with the surrounding vegetation facilitate the movement of crawling herbivores between different plant individuals. Herbivore-induced VOC emission may not only attract plant defenders to the sender, but also to closely neighboured Lima bean plants which receive the information and subsequently secrete increased amounts of EFN. KOBAYASHI and YAMAMURA (2003) suggested the term ‘‘cooperative signal’’ to describe such an interaction between undamaged and herbivore infested plant individuals that signal together as if they were collectively calling for bodyguards. A potential conflict of interests between sender and receiver is solved when the benefit of the emitted VOCs (i.e. effective reduction of the herbivore pressure within a patch) is counterbalanced by the physiological and ecological costs for the VOC-emitting plant (SABELIS & DE JONG, 1988). The same should apply when sender and receiver belong to different species and share the same phytophages (BRUIN & DICKE, 2001). However, the situation reverses, if sender and receiver (Lima bean) belong to different species, yet the sender is attacked by a specialist that would not attack the receiver of the signal. In this case, the receiver would pay the costs of activating a defence without an actual need. Our results have shown that increased amounts of EFN, which were either applied experimentally or induced by airborne VOCs, benefited Lima bean under natural growing conditions (Manuscript II, III and IV).

If the Lima bean would have benefited of increased amounts of EFN, why did it not activate this defence by itself? The answer to this interesting question cannot be answered based on the data available. A conceivable explanation could be that the ecological strategy of the liana Lima bean is to grow rather than to defend and that this strategy is more successful on longer time-scales (i.e. when more than one generation is regarded). The level of VOCs which have been applied experimentally to tendrils (Manuscript III and IV) may have exceeded the amount of VOCs, to which a Lima been tendril is naturally exposed. If corresponding amounts of VOCs reach a Lima bean tendril, it would allocate more resources to defence. This could be regulated via a certain threshold which is needed to activate EFN secretion.

Chemical composition and specificity of the VOC blend

Precise knowledge on the qualitative and quantitative composition of the emitted VOC blends is a prerequisite for understanding their role for mediating complex interactions between different trophic levels. The example in Manuscript V has shown that two monoterpenes, which previously have been considered as constituents of the induced VOC blend of Lima bean (ENGELBERTH *et al.*, 2001; HEIL, 2004a), could now be identified as degradation products resulting from a chemical reaction with the adsorption materials used for volatile-trapping. This finding highlights the need to always take artefact formation into account when using standard methods for headspace sampling (BRUNKE *et al.*, 1993).

Beyond, it remains elusive whether the two observed compounds are pure artefacts of the sampling methods or whether they are also occasionally formed by the Lima bean under natural conditions. Such a reaction could proceed either actively by involving enzymatic activities or passively under the influence of certain abiotic factors or the catalytic activity of the plant surface. In the latter case, the question arises how the transmission of informative airborne signals can be maintained despite interfering processes like the one described in manuscript V. The very first step to answer this question would be to analyse whether the two described artefacts are also formed under natural conditions and if so, which factors favour their formation.

Besides this reaction for which it is not yet clear whether it also plays a role under natural conditions, several other factors hamper a reliable interpretation of the emitted VOCs. For example, the VOC profile emitted from Lima bean plants upon herbivore damage is subject to a considerable variability both on the quantitative and qualitative level (Manuscript I). Causal for this variation is a complex interplay of numerous factors such as time of day, leaf growth stage, species and life stage of the attacking herbivore as well as abiotic conditions (e.g. light and water availability; TAKABAYASHI *et al.*, 1994a). In its natural environment, Lima bean is surrounded by many other plant species which also emit highly variable blends of VOCs, not only to attract carnivores, but also pollinators. For example, the eight most abundant VOCs emitted from the Lima bean are very common constituents of the herbivore-induced VOC plumes of many different plant species (Tab. 1). Thus, the receiver of the emitted volatile signals, no matter if insect or neighbouring plant, has to cope with these difficulties.

In the case of arthropods, evidence that mixing of odour blends interferes with host plant localization is controversial. Among the few studies that addressed this issue, some showed masking for herbivores (VISSER, 1986; VISSER & AVÉ,

1978) and carnivores (MONTEITH, 1960; SHIOJIRI *et al.*, 2000; VOS *et al.*, 2001), whereas others did not find evidence of such antagonistic effects (DICKE *et al.*, 2003b; RÖTTGER, 1979). The detectability of the emitted cue depends most likely on the amount of compounds shared with the interfering blend, the tuning of the insect receptors to specific compounds as well as the wind speed within a given habitat (VISSER, 1986). Associative learning is one possible strategy arthropods have developed to cope with these difficulties (PAPAJ & LEWIS, 1993; VET *et al.*, 1995).

The situation may be different for plant-plant communication. Insects often need to be attracted from larger distances. In contrast, for 'eavesdropping' plants it is more important to sense the immediate vicinity for cues indicating an increased risk of herbivory. Plants should respond to single or few compounds that are emitted rapidly after tissue damage in a sharp dosage-dependent manner, rather than to highly variable mixtures of different VOCs. Indeed our results are consistent with these predictions. Despite our artificial VOC mixture mimicked the quantitative and qualitative composition of a naturally induced blend as to emission within 24 h, our mode of application may not have perfectly matched the natural situation at a given time point within this period (Manuscript III). The fact that we observed significantly increased EFN secretion rates despite this inevitable systematic error suggests that the absence or presence of certain compounds (such as (*Z*)-3-hex-3-enyl acetate) is responsible for the effect rather than the relative composition of the whole blend. Preliminary trials for the EFN-induction experiments (Manuscript III) indicated that a certain threshold of VOCs is required to reliably induce EFN secretion (results not shown), thereby providing first hints to a dose-response relationship between airborne VOCs and the amount of secreted EFN. More experiments are definitely needed in which combinations of different compounds in varying concentrations are tested for their EFN-inducing effect in Lima bean and other plant species.

The role of EFN for plant defence

The hypothesis of an adaptive function of extrafloral nectaries providing safeguards from herbivores is much better supported by field studies (for review see BEATTIE, 1985; BENTLEY, 1977a) than the indirect defence hypothesis of VOCs. The specificity that is needed for a volatile signal to attract plant defenders from longer distances does presumably not apply to EFNs. Due to the reward-based nature of this signal, the open presentation of a carbohydrate-rich food source itself is sufficient to attract foraging ants or other plant visitors from short distances. In the Lima bean, the mere application of an artificial EFN mixture that mimicked the natural

blend both quantitatively and qualitatively attracted natural enemies of the herbivores (Manuscript II, III and IV). In this context it would be interesting to see whether the qualitative composition of Lima bean EFN changes in response to different herbivores and whether these changes can alter the composition of the attracted arthropod community (BLÜTHGEN & FIEDLER, 2004).

An increase in the availability of EFN attracted more putative plant defenders to Lima bean plants (Manuscript II). In the long run (i.e. after 25 d), experimentally increasing the amounts of EFN had a positive effect on plant fitness as compared to controls (Manuscript III and IV). A great diversity of arthropods was attracted to both EFN-treated and control tendrils (Manuscript II). Furthermore, the positive effects of the nectar treatment counterbalanced any negative effects associated with this defence, such as an attraction of additional herbivores or hyper-parasitoids. Thus in Lima bean, EFN can be considered a functioning indirect defence mechanism.

Ants as plant defenders

The secretion of EFN represents a loose form of facultative mutualism that attracts a broad spectrum of different arthropods. Among them, ants are most dominant. The attraction of ants through extrafloral nectaries acting as defensive agents has been suggested as early as 1874 (DELPINO cited in VON WETTSTEIN, 1889). Since then, a growing body of research has revealed ant-mediated plant protection (for review see BENTLEY, 1977a; BUCKLEY, 1982; HEIL & MCKEY, 2003; KOPTUR, 1992). Also the presented field experiments were consistent with these findings: ants were by far the most dominant arthropod groups attracted to Lima bean tendrils thereby being most likely responsible for the observed enhancement of plant protection (Manuscript II, III and IV). The ecological dominance of ants may be caused by their territoriality that facilitates monopolisation of food sources (HÖLLDOBLER & LUMSDEN, 1980). Furthermore, their capability of learning (HARRISON & BREED, 1987) in combination with a foraging strategy that involves frequent patrolling, efficient recruitment and trail laying allows a highly optimised exploitation of rewarding food sources (HÖLLDOBLER & WILSON, 1990). Indeed, behavioural observations suggested a defence-by-exploitation strategy (DREISIG, 2000): resident generalist ant species avoided competition with other EFN-consumers by increasing the visitation frequency of the extrafloral nectaries. The increased presence of these ants on plants with higher amounts of EFN facilitated the defensive effect (Manuscript II).

Wasps and flies as putative plant defenders

Not all studies on the facultative mutualism between ants and EFN-secreting plants detected measurable beneficial effects on the plant partner (BECERRA & VENABLE, 1989; BOECKLEN, 1984; O'DOWD & CATCHPOLE, 1983; RASHBROOK *et al.*, 1992). In the majority of these studies, this issue was addressed by excluding ants using sticky barriers such as Tangletrap® (BARTON, 1986; MODY & LINSENMAIR, 2004; O'DONNELL *et al.*, 1996). The drawback of this attempt is that putative flying plant defenders remain unaffected by this approach. Given that - under certain conditions - these are more important for plant defence than ants, no protective effect would be detected with such an experimental design, since the flying plant defenders would have had access to both ant-exclusion and control plants. By manipulating the amount of available EFN, we could circumvent these difficulties and demonstrate a beneficial effect of EFN on the Lima bean (Manuscript II and IV). The increased abundance of predaceous and parasitoid insects such as flies and wasps suggests that besides ants, also these flying defenders may have contributed to increasing the plant's fitness (Manuscript II).

Captures with sticky traps indicated an enormous diversity of dipteran and hymenopteran families visiting the Lima bean plants (Manuscript II and IV). These findings suggest that *P. lunatus* does not exclusively rely on a highly specialised interaction with a reduced number of defending species or taxa, but is rather loosely associated with a broad range of different organisms. For many of which it is not yet clear whether they have a positive, negative or neutral effect on the plant. A high diversity of arthropods visiting the Lima bean may further hint to the absence of regulatory mechanisms of EFN quality, as they are known from obligate ant-plant-mutualisms, which act as a filter to exclude less desirable partners in multi-species associations (HEIL *et al.*, 2005).

Predators versus parasitoids

Members of the third trophic level which are attracted by indirect defences such as HI-VOCs or EFN can be either predators or parasitoids. Predators kill their herbivorous prey immediately, thus directly preventing further damage to the plant. The benefit for the plant is less obvious in the case of parasitoids. These lay their eggs on or in the herbivore, thus killing their host after hatching of their larvae (STEIDLE & VAN LOON, 2002). Depending on the parasitoid species, egg laying can reduce or increase the feeding and growth of their herbivorous host (HARVEY, 2000; VAN ALPHEN & JERVIS, 1996). Consequently, the benefit for the plant depends strongly on which plant defenders are attracted to VOCs or EFN. The defence

strategy of the Lima bean relied predominantly on ants, with other putative plant defenders like non-ant predators or parasitoid species playing a minor role (Manuscript II, III and IV). A proximate reason for this observation could be that the liana-like growth structure, which is characterized by a close contact to the surrounding vegetation, may favour ants as plant-defending agents, since they can be easily attracted in larger numbers from the vicinity. In contrast, the foraging movement of flying defenders could be hindered by the intricate growth architecture of the plant (PRICE *et al.*, 1980). Another explanation could be the time-scales at which ant- and parasitoid-mediated plant defence operates at optimal effectiveness: Due to the relatively short vegetation periods of the Lima bean at the experimental sites, ants may be more effective, because a beneficial effect resulting from parasitoid defence requires a longer time.

Benefit of indirect defences depends on the ecological context

Interestingly, the numbers of attracted arthropods varied considerably between two sites, resulting in a spatial variation of the protective effect (Manuscript II). Similar observations have been reported for other EFN-secreting plant species (BENTLEY, 1976; COGNI *et al.*, 2003; HORVITZ & SCHEMSKE, 1984; INOUYE & TAYLOR, 1979). Hence, in facultative ant-plant associations, the benefit for the EFN-secreting plant appears to be directly linked to the availability of potential plant defenders. Therefore, the performance of Lima bean within different habitat types is likely constrained by the local abundance of beneficial insects.

Plants located within such a mosaic of environments with a varying presence of plant defenders adapt their defensive phenotype according to the different conditions. If nectar feeders are absent, EFN secretion has been reported to decline (HEIL *et al.*, 2000b). The production rates could be partially restored again when insects gained access to the EFN-secreting plant. Similar responses are known for the production of floral nectars (GILL, 1988; KOPTUR, 1983; PYKE, 1991). In some plant species, nectars are even reabsorbed in flowers where insect visitation has been prevented (BÚRQUEZ & CORBET, 1991; FAHN, 1988). So far, the regulatory mechanisms underlying these processes are unknown. However, such economy in the regulation of food rewards may save a plant metabolic energy which can be invested in alternative plant traits. Direct defence strategies for example may be increasingly activated when insect plant defenders are absent.

9. Synthesis

The chapters compiled in this thesis encompass analyses on induced, indirect plant defences against herbivory and their role for mediating multispecies interactions. The impetus for these studies was to transfer the knowledge on this issue, which mainly has been gathered in extensive laboratory experiments, to the field, and to verify its applicability to wild growing plant populations of the model plant Lima bean. Field experiments at the plant's natural growing site yielded results that could answer some of the initial questions, yet several surprising observation stimulated also several new hypotheses.

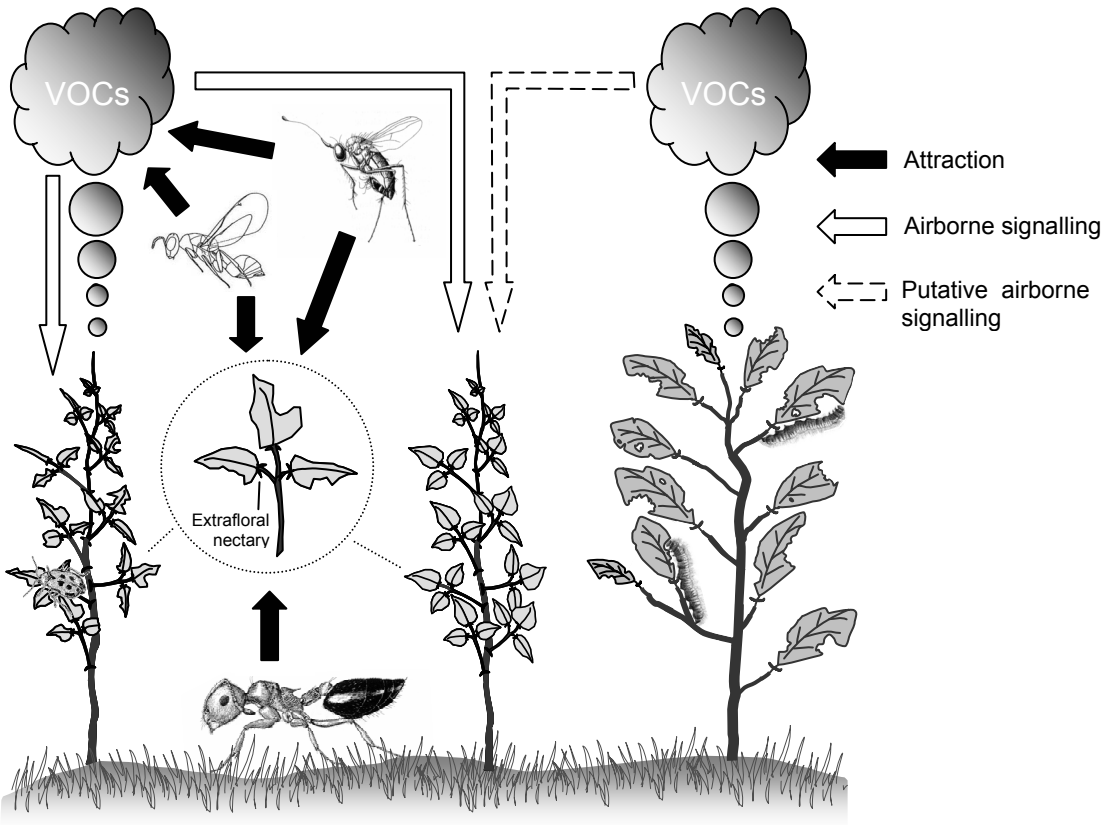


Figure 1. The role of induced, indirect defences for mediating intra- and interspecific interactions in the *Phaseolus lunatus* system. In the Lima bean (left plant), herbivory induces the secretion of EFN and the emission of VOCs. Both cues attract wasps and flies. Ants are mainly attracted to EFN. Beyond, VOCs are also implicated in intra-individual and intra-specific plant-plant communication. Parts of the same (left) or neighbouring plants (middle) induce the secretion of EFN in response to airborne VOCs emitted from herbivore-damaged plants or plant-parts (left). Also VOC blends emitted from heterospecific plants (right) which overlap in their qualitative composition with the one emitted from Lima bean may induce defence responses in eavesdropping Lima bean plants. Sources: Ant (*Crematogaster laeviscula* var. *clara* MAYR (SMITH, 1947); caterpillar (*Macrothylia rubi* LINNAEUS; HONOMICHL et al., 1996); wasp (*Austrotoxeuma kuscheli*; NOYES, 2003), beetle (*Cerotoma trifurcata* FORSTER; http://ipm.ncsu.edu/AG295/html/bean_leaf_beetle.htm), fly (*Ludovicius eucerus* LOEW; SÉGUY, 1951).

In the following, the gathered results are merged to develop a theoretical model which may serve as a basis for future examinations (see also Fig. 1):

(1) In the Lima bean (*Phaseolus lunatus*) herbivory induces both the emission of VOCs as well as the secretion of EFN.

(2) Among these two indirect defences, EFN secretion is the more important defence mechanism for the Lima bean. The reward-based nature of this signal attracts – depending on their availability in different environments – a broad spectrum of flying and crawling arthropods with varying defensive capabilities. Ants are likely to be most important for plant defence, but also carnivorous wasps, flies and also other taxonomic groups may contribute to prevent herbivory. Increasing the amount of secreted EFN can benefit the Lima bean in terms of an enhanced reproductive success.

(3) The primary function of VOCs, which are increasingly emitted upon herbivore damage, is the intra-individual and intra-specific conveyance of information on a currently increased risk of being attacked. Lima bean tendrils receiving this cue cautionary increase their secretion rate of EFN, thereby benefiting from receiving this signal.

(4) A secondary function of VOCs is the attraction of members of the third trophic level which can associate the complex information encoded in the emitted VOC plumes with the emitting plant species as well as with the abundance and species of the attacking herbivore. Also the attraction of predacious or parasitoid plant defenders benefits the VOC-emitting plant.

(5) (*Z*)-3-hex-3-enyl acetate is one signal (possibly among others) that conveys the information on an impending herbivore attack to yet undamaged Lima bean tendrils. In face of the wide taxonomic distribution of the ability of plants to emit this compound, it appears reasonable to assume that the sender may not only be downwind-located tendrils of the same or other Lima bean individuals but also heterospecific plants attacked by generalist herbivores come into question as putative senders.

10. Summary

10.1. Summary

Due to their sessile nature of growth, which does not allow them to escape unfavourable situations, plants have evolved a plethora of mechanisms to cope with various abiotic and biotic stress factors. The defensive mechanisms against herbivory, for example, cover a diverse spectrum of mechanical barriers such as thorns or hairs (trichomes), and chemical defences that are toxic to the attacking organism or reduce the nutritional value of the plant material. Such defence strategies, which directly wield a negative impact on the attacking herbivore, have been termed *direct* defences. In contrast, *indirect* defences recruit members of the third trophic level, which reduce the number of currently feeding herbivores. Attraction of beneficial arthropods to plants can be mediated by herbivore-induced plant volatiles (HIPVs), the provision of shelter in so-called domatia, or by plant-provided food supplements such as extrafloral nectar (EFN). Direct and indirect defences can either be expressed constitutively (i.e. permanently) or be induced in response to herbivore attack.

The Lima bean (*Phaseolus lunatus*) features the emission of volatile organic compounds (VOCs) and the secretion of EFN. Both indirect defences are inducible upon herbivore damage as well as after treatment with the phytohormone jasmonic acid (JA). Using Lima bean as a model plant, several studies are available to date on mechanistic and functional aspects of herbivore-induced VOC emission applying ecological, analytical and molecular approaches. However, the secretion of EFN has largely been neglected as an alternative defensive strategy of *Phaseolus lunatus*. Research on indirect defences of the Lima bean has been mainly conducted under laboratory conditions, thus evidence for the ecological role of these two indirect defences in nature is largely lacking.

The aim of the present thesis was to study the function of the two indirect defences - emission of HIPVs and secretion of VOCs - at the plant's natural growing site, and to unravel their role for mediating intra- and interspecific interactions. A review on the mechanisms of herbivore recognition, signal transduction, the biosynthesis of HIPVs as well as ecological and evolutionary aspects of induced, indirect defences summarized already existing knowledge. Field experiments on plants growing in a native population near Puerto Escondido (Oaxaca, Mexico) were conducted to investigate whether EFN secretion and VOC emission benefit Lima bean at its natural growing site, to assess the arthropod community which is attracted to VOCs and EFN, to scrutinize whether HIPVs are involved in plant-plant communication, and to contrast the defensive role of EFN to that of VOCs. Laboratory experiments were performed on the formation of artefacts which have been ob-

served during headspace sampling of induced Lima bean plants.

HIPVs induced the secretion of EFN in neighbouring Lima bean tendrils. Volatiles released from herbivore-damaged bean tendrils as well as an artificial volatile blend resembling the naturally released blend elicited the indirect defence in treated tendrils. A single-compound comparison of the eight most important VOCs within the herbivore-induced blend identified the green leaf volatile (*Z*)-3-hex-3-enyl acetate as being responsible for the observed defence reaction. These results suggest that plant-plant communication between different tendrils of the same or of several plant individuals occurs in Lima bean.

Increased amounts of EFN and VOCs benefited Lima bean plants in nature. In a long-term experiment designed to disentangle the defensive effects of VOCs and EFN, tendrils sprayed with jasmonic acid (JA) to induce both indirect defences were compared to tendrils treated with an artificial blend of either EFN or VOCs as well as to control tendrils. Repeated application of EFN and VOCs benefited the experimental tendrils as indicated by increased plant fitness correlates. Because tendrils treated with JA showed a similar yet weaker response, it is concluded that a putative JA-dependent direct defence was of minor importance, since otherwise JA-treated tendrils should have performed better than VOC- and EFN-treated tendrils. VOC- and JA-treated tendrils had produced similar amounts of EFN. However, tendrils treated with synthetic EFN only benefited equally or more of this treatment than did tendrils treated with VOCs or JA. This finding indicates that - under our experimental conditions - EFN had played a more important role as an indirect defence for Lima bean than did a VOC-mediated arthropod attraction.

Ants were the most important plant defenders. Treatment of Lima bean tendrils with JA or an artificial blend of either VOCs or EFN significantly increased the number of ants and wasps as compared to controls. The numbers of flies visiting the experimental tendrils were significantly increased only on tendrils treated with artificial EFN. However, ants were ca. 25-fold more abundant than wasps and ca. 10-fold more frequently observed than flies. Sticky traps attached to the experimental tendrils trapped predominantly Diptera and Hymenoptera (55 % and 26 % of all insects trapped). The observation that 98 % of all hymenopterans and 77 % of all dipterans belonged to families with parasitoid or predacious life habits indicates that besides ants and wasps also flies may have contributed to the observed beneficial effect.

Active carbon, which is frequently used as adsorption material during headspace sampling, dehydrogenates ocimene and thus leads to artefact formation. During headspace sampling of JA-induced Lima bean plants we observed the two monoterpenes (3*E*,5*E*)-2,6-dimethyl-3,5,7-octatrien-2-ol and (3*E*,5*E*)-2,6-dimethyl-1,3,5,7-octatetraene only if volatiles were trapped using activated charcoal, but not with alternative adsorption materials such as SPME. These two substances could be identified as artefacts derived from (*E*)-β-ocimene. Based on further experiments, the involvement of radicals could be excluded. Instead, a mechanism is proposed in which the ocimene is dehydrogenated by interactions with the surface of active carbon in a DDQ-like dehydrogenation reaction. The catalytic capacity of the active carbon could be further exploited for a rapid and efficient functionalisation of ocimene to yield (3*E*,5*E*)-2-alkoxy-3,5,7-octatrienes using methanol, ethanol, or isopropanol as solvents.

10.2 Zusammenfassung

Aufgrund ihrer sessilen Lebensweise sind Pflanzen nicht dazu in der Lage, ungünstigen Bedingungen aktiv auszuweichen. Im Laufe der Evolution haben sie daher eine Vielzahl von Abwehrreaktionen entwickelt, um auf die verschiedensten biotischen und abiotischen Umweltfaktoren reagieren zu können. Verteidigungsstrategien gegen Herbivore zum Beispiel umfassen ein breites Spektrum, welches von mechanischen Barrieren wie Dornen oder Haaren (Trichome) bis hin zu chemischen Abwehrmechanismen reicht. Letztere wirken entweder giftig auf den angreifenden Organismus oder reduzieren die Verwertbarkeit des pflanzlichen Materials. Solche Abwehrstrategien, die einen direkt negativen Einfluss auf den angreifenden Herbivoren ausüben, werden unter der Bezeichnung *direkte* Abwehrmechanismen zusammengefasst. Im Gegensatz dazu locken *indirekte* Pflanzenverteidigungen Vertreter der dritten trophischen Stufe an, die dann die Anzahl der angreifenden Herbivoren reduzieren. Eine solche Anlockung von für die Pflanze nützlichen Arthropoden kann z.B. durch leicht flüchtige organische Verbindungen (Duftstoffe) erreicht werden, welche nach Herbivorenbefall vermehrt produziert werden. Alternative Möglichkeiten sind die Bereitstellung von Unterschlupfmöglichkeiten in so genannten Domatien, oder das Anbieten attraktiver Nahrungsquellen wie z.B. extrafloraler Nektar. Direkte und indirekte Verteidigungsformen können entweder konstitutiv (d.h. permanent) ausgebildet sein, oder erst durch Herbivorenbefall induziert werden.

Die Limabohne (*Phaseolus lunatus*) emittiert Duftstoffe und sezerniert extrafloralen Nektar. Beide indirekten Verteidigungen sind sowohl durch Herbivorie als auch durch Behandlung mit dem Pflanzenhormon Jasmonsäure (JA) induzierbar. In mehreren ökologischen, analytischen oder molekularen Studien konnten bereits verschiedene mechanistische und funktionelle Aspekte der im Modellsystem Limabohne durch Herbivorbefall induzierten Duftstoffemission aufgeklärt werden. Im Gegensatz dazu wurde die Sekretion extrafloralen Nektars als alternative Verteidigungsstrategie von *Phaseolus lunatus* bisher kaum beachtet. Die bisher durchgeführten Untersuchungen zur indirekten Verteidigung der Limabohne wurden fast ausschließlich unter Laborbedingungen durchgeführt, weswegen Hinweise auf die ökologische Rolle der beiden indirekten Verteidigungen unter Freilandbedingungen bisher fehlen.

Die Zielsetzung der vorliegenden Arbeit war es daher, die Funktion der beiden indirekten Verteidigungen, Duftstoffemission und Nektarsekretion, am natürlichen Standort der Pflanze zu untersuchen, sowie deren Rolle für die Vermittlung intra- und interspezifischer Interaktionen zu analysieren. Hierzu wurde zunächst der derzeitige Kenntnisstand zu den Mechanismen der Herbivorenerkennung, Signalwei-

terleitung, Duftstoffbiosynthese sowie ökologischen und evolutionären Aspekten der beiden indirekten Verteidigungen in einem Übersichtsartikel zusammengefasst. Darüber hinaus wurden Feldexperimente an einer natürlich wachsenden, westlich von Puerto Escondido (Oaxaca, Mexiko) lokalisierten Population von Limabohnen durchgeführt. Hierbei sollte der jeweils aus Duftstoffemission und Sekretion extrafloralen Nektars für die Limabohne resultierende Vorteil quantifiziert, und einander vergleichend gegenübergestellt werden. Zusätzlich wurde die von extrafloralem Nektar oder Duftstoffen angelockte Arthropodengemeinschaft erfasst, sowie die Rolle der Duftstoffe für zwischenpflanzliche Kommunikation untersucht. In Laborexperimenten wurde die bei der Duftstoffsammlung induzierter Limabohnen beobachtete Artefaktbildung analysiert.

Duftstoffe induzierten die Sekretion extrafloralen Nektars in benachbarten Ranken der Limabohne. Die indirekte Verteidigung konnte sowohl von Duftstoffen ausgelöst werden, die von durch Herbivoren beschädigten Ranken emittiert wurden, als auch von einer künstlichen Mischung, deren Zusammensetzung das natürlich emittierte Duftstoffspektrum nachahmte. Bei einem Einzelvergleich der acht wichtigsten Bestandteile des Limabohnenduftspektrums konnte (*Z*)-3-Hex-3-enyl acetat als Auslöser für die beobachtete Verteidigungsreaktion identifiziert werden. Diese Befunde weisen auf eine „Kommunikation“ zwischen verschiedenen Ranken desselben oder mehrerer unterschiedlicher Pflanzenindividuen der Limabohne hin.

Die Limabohne profitierte unter Freilandbedingungen von einer erhöhten Menge an extrafloralem Nektar und Duftstoffen. In einem Langzeitexperiment sollte die Verteidigungswirkung von Duftstoffen und extrafloralem Nektar experimentell getrennt werden. Hierzu wurden Bohnenranken mit Jasmonsäure besprüht um beide indirekte Verteidigungen zu aktivieren. Diese wurden dann sowohl mit Kontrollranken als auch mit Ranken verglichen, die mit einer künstlichen Mischung aus entweder Duftstoffen oder extrafloralem Nektar behandelt worden waren. Wiederholte Behandlung von Ranken mit Duftstoffen oder extrafloralem Nektar verbesserte die Entwicklung fitness-relevanter Pflanzenparameter, was auf einen aus beiden indirekten Verteidigungen resultierenden Vorteil für die Pflanze deutet. Mit Jasmonsäure behandelte Ranken zeigten einen ähnlichen, jedoch schwächeren Effekt. Dies deutet darauf hin dass eine eventuell durch Jasmonsäure induzierte direkte Verteidigung eine untergeordnete Rolle zu spielen scheint, da sich mit Jasmonsäure behandelte Ranken sonst hätten besser entwickeln müssen als die mit Duftstoffen oder extrafloralem Nektar behandelten Ranken.

Jasmonsäure- und Duftstoffbehandlung führte zu einer erhöhten Sekretionsrate extrafloralen Nektars, wobei keine quantitativen Unterschiede zwischen beiden Behandlungsgruppen festgestellt werden konnten. Ranken, auf die künstlicher extrafloraler Nektar aufgebracht worden war, profitierten genauso stark oder stärker von ihrer Behandlung als mit Duftstoffen oder Jasmonsäure behandelte Ranken. Dieser Befund deutet darauf hin, dass unter unseren experimentellen Bedingungen extrafloraler Nektar eine wichtigere Rolle als indirekte Pflanzenverteidigung spielte als eine Duftstoff-vermittelte Anlockung von Arthropoden

Ameisen waren die wichtigsten Pflanzenverteidiger. Die Behandlung von Limabohnenranken mit Jasmonsäure oder der künstlichen Duftstoff- oder Nektarmischung führte im Vergleich zu Kontrollranken zu einer signifikant erhöhten Anzahl angelockter Ameisen und Wespen. Die Zahl der angelockten Fliegen war nur bei den mit künstlichem extrafloralem Nektar behandelten Ranken signifikant erhöht. Generell wurden Ameisen etwa 25x häufiger als Wespen und ca. 10x häufiger als Fliegen beobachtet. In einer Erfassung der zur Limabohne angelockten Insekten mit Klebefallen wurden hauptsächlich Diptera und Hymenoptera (55 und 26 % aller gefangener Insekten) gefangen. Die Tatsache dass 98 % aller gefangenen Hymenopteren und 77 % aller Dipteren parasitoidisch oder räuberisch lebenden Familien angehörten, deutet darauf hin, dass neben Wespen und Ameisen auch die angelockten Fliegen einen Beitrag zur Verteidigung der Limabohne geleistet haben könnten.

Aktivkohle, ein häufig bei der Duftstoffsammlung verwendetes Adsorptionsmaterial dehydriert Ocimen und führt so zur Artefaktbildung. Bei der Duftstoffsammlung von mit Jasmonsäure induzierter Limabohnen konnten wir die beiden Monoterpene (3*E*,5*E*)-2,6-Dimethyl-3,5,7-oktatrien-2-ol und (3*E*,5*E*)-2,6-Dimethyl-1,3,5,7-oktatetraen nur beobachten, wenn Aktivkohle, nicht aber wenn alternative Adsorptionsmaterialien wie z.B. SPME, verwendet wurden. Die beiden Verbindungen konnten als von (*E*)-β-Ocimen abgeleitete Artefakte identifiziert werden. Weitere Experimente deuteten darauf hin, dass die bei der Artefaktbildung beteiligte chemische Reaktion nicht über eine radikalische Zwischenstufe verläuft. Stattdessen wird ein Mechanismus vorgeschlagen, bei dem Ocimen in Wechselwirkung mit der Aktivkohlenoberfläche in einem DDQ-ähnlichen Reaktionsmechanismus dehydriert wird. Die entsprechenden Reaktionseigenschaften der Aktivkohle konnten darüber hinaus zu einer schnellen und effizienten Funktionalisierung von Ocimen zu (3*E*,5*E*)-2-Alkoxy-3,5,7-octatrienen mit Methanol, Ethanol oder Isopropanol als Lösungsmittel genutzt werden.

11. References

ADLER FR & KARBAN R (1994) Defended fortresses or moving targets? Another model of inducible defenses inspired by military metaphors. *The American Naturalist*, **144**, 813-832.

AGRAWAL AA (2000) Host-range evolution: Adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology*, **81**, 500-508.

AGRAWAL AA & KARBAN R (1997) Domatia mediate plant-arthropod mutualism. *Nature*, **387**, 562-563.

AGRAWAL AA & DUBIN-THALER BJ (1999) Induced responses to herbivory in the Neotropical ant-plant association between *Azteca* ants and *Cecropia* trees: Response of ants to potential inducing cues. *Behavioral Ecology and Sociobiology*, **45**, 47-54.

AGRAWAL AA, CONNER JK, JOHNSON MTJ & WALLSGROVE R (2002a) Ecological genetics of an induced plant defense against herbivores: Additive genetic variance and costs of phenotypic plasticity. *Evolution*, **56**, 2206-2213.

AGRAWAL AA, JANSSEN A, BRUIN J, POSTHUMUS MA & SABELIS MW (2002b) An ecological cost of plant defence: Attractiveness of bitter cucumber plants to natural enemies of herbivores. *Ecology Letters*, **5**, 377-385.

AHARONI A, GIRI AP, DEUERLEIN S, GRIEPINK F, DE KOGEL WJ, VERSTAPPEN FWA, VERHOEVEN HA, JONGSMA MA, SCHWAB W & BOUWMEESTER HJ (2003) Terpenoid metabolism in wild-type and transgenic *Arabidopsis* plants. *Plant Cell*, **15**, 2866-2884.

ALBORN T, TURLINGS TCJ, JONES TH, STENHAGEN G, LOUGHRIN JH & TUMLINSON JH (1997) An elicitor of plant volatiles from beet armyworm oral secretion. *Science*, **276**, 945-949.

ALMÉRAS E, STOLZ S, VOLLENWEIDER S, REYMOND P, MENE-SAFFRANE L & FARMER EE (2003) Reactive electrophile species activate defense gene expression in *Arabidopsis*. *Plant Journal*, **34**, 205-216.

ARIMURA GI, HUBER DPW & BOHLMANN J (2004a) Forest tent caterpillars (*Malacosoma disstria*) induce local and systemic diurnal emissions of terpenoid volatiles in hybrid poplar (*Populus trichocarpa* x *deltoides*): cDNA cloning, functional characterization, and patterns of gene expression of (-)-germacrene D synthase, *PtdTPS1*. *Plant Journal*, **37**, 603-616.

ARIMURA GI, KOST C & BOLAND W (2005) Herbivore-induced, indirect plant defences. *Biochimica et Biophysica Acta - Lipids and Lipid Metabolism*, **1734**, 91-111.

ARIMURA GI, OZAWA R, HORIUCHI J, NISHIOKA T & TAKABAYASHI J (2001) Plant-plant interactions mediated by volatiles emitted from plants infested by spider mites. *Biochemical Systematics and Ecology*, **29**, 1049-1061.

ARIMURA GI, OZAWA R, KUGIMIYA S, TAKABAYASHI J & BOHLMANN J (2004b) Herbivore-induced defense response in a model legume. Two-spotted spider mites induce emission of (*E*)-β-ocimene and transcript accumulation of (*E*)-β-ocimene synthase in *Lotus japonicus*. *Plant Physiology*, **135**, 1976-1983.

ARIMURA GI, OZAWA R, SHIMODA T, NISHIOKA T, BOLAND W & TAKABAYASHI J (2000a) Herbivory-induced volatiles elicit defence genes in Lima bean leaves. *Nature*, **406**, 512-515.

ARIMURA GI, TASHIRO K, KUHARA S, NISHIOKA T, OZAWA R & TAKABAYASHI J (2000b) Gene responses in bean leaves induced by herbivory and by herbivore-induced volatiles. *Biochemical and*

Biophysical Research Communications, **277**, 305-310.

ARIMURA GI, OZAWA R, NISHIOKA T, BOLAND W, KOCH T, KUHNEMANN F & TAKABAYASHI J (2002) Herbivore-induced volatiles induce the emission of ethylene in neighboring Lima bean plants. *Plant Journal*, **29**, 87-98.

ARNETT RH (2000) *American insects - A handbook of the insects of America north of Mexico*. CRC Press, Boca Raton.

ARTHUR CL & PAWLISZYN J (1990) Solid-phase microextraction with thermal-desorption using fused-silica optical fibers. *Analytical Chemistry*, **62**, 2145-2148.

AUBOURG S, LECHARNY A & BOHLMANN J (2002) Genomic analysis of the terpenoid synthase (*AtTPS*) gene family of *Arabidopsis thaliana*. *Molecular Genetics and Genomics*, **267**, 730-745.

AVÉ DA, GREGORY P & TINGEY WM (1987) Aphid repellent sesquiterpenes in glandular trichomes of *Solanum berthaultii* and *Solanum tuberosum*. *Entomologia Experimentalis et Applicata*, **44**, 131-138.

BAKER HG, OPLER PA & BAKER I (1978) A comparison of the amino-acid complements of floral and extrafloral nectars. *Botanical Gazette*, **139**, 322-332.

BALDWIN IT (1988) The alkaloidal responses of wild tobacco to real and simulated herbivory. *Oecologia*, **77**, 378-381.

BALDWIN IT (2001) An ecologically motivated analysis of plant-herbivore interactions in native tobacco. *Plant Physiology*, **127**, 1449-1458.

BALDWIN IT & SCHULTZ JC (1983) Rapid changes in tree leaf chemistry induced by damage: Evidence for communication between plants. *Science*, **221**, 277-279.

BALDWIN IT & CALLAHAN P (1993) Autotoxicity and chemical defense - Nicotine accumulation and carbon gain in solanaceous plants. *Oecologia*, **94**, 534-541.

BALDWIN IT & PRESTON CA (1999) The eco-physiological complexity of plant responses to insect herbivores. *Planta*, **208**, 137-145.

BALDWIN IT & HAMILTON W (2000) Jasmonate-induced responses of *Nicotiana sylvestris* results in fitness costs due to impaired competitive ability for nitrogen. *Journal of Chemical Ecology*, **26**, 915-952.

BALDWIN IT, SCHMELZ EA & OHNMEISS TE (1994) Wound-induced changes in root and shoot jasmonic acid pools correlate with induced nicotine synthesis in *Nicotiana sylvestris* Spegazzini and Comes. *Journal of Chemical Ecology*, **20**, 2139-2157.

BALDWIN IT, KESSLER A & HALITSCHKE R (2002) Volatile signaling in plant-plant-herbivore interactions: What is real? *Current Opinion in Plant Biology*, **5**, 351-354.

BALLHORN DJ, LIEBEREI R & GANZHORN JU (2005) Plant cyanogenesis of *Phaseolus lunatus* and its relevance for herbivore-plant interaction: The importance of quantitative data. *Journal of Chemical Ecology*, **31**, 1445-1473.

BARATA EN, PICKETT JA, WADHAMS LJ, WOODCOCK CM & MUSTAPARTA H (2000) Identification of host and nonhost semiochemicals of eucalyptus woodborer *Phoracantha semipunctata* by gas chromatography-electroantennography. *Journal of Chemical Ecology*, **26**, 1877-1895.

BARGONI N (1972) Synthesis of sucrose in the nectary of *Convolvulus sepium*. *Bollettino Societa Italiana Biologia Sperimentale*, **48**, 1159-1160.

BARTON AM (1986) Spatial variation in the effect of ants on an extrafloral nectary plant. *Ecology*, **67**, 495-504.

BATE NJ & ROTHSTEIN SJ (1998) C_6-volatiles derived from the lipoxygenase

pathway induce a subset of defense-related genes. *Plant Journal*, **16**, 561-569.

BEATTIE AJ (1985) *The evolutionary ecology of ant-plant mutualisms*. Cambridge University Press, Cambridge.

BECERRA JXI & VENABLE DL (1989) Extrafloral nectaries: A defense against ant-Homoptera mutualisms? *Oikos*, **55**, 276-280.

BELL E, CREELMAN RA & MULLET JE (1995) A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 8675-8679.

BENNETT RN & WALLSGROVE RM (1994) Tansley Review No. 72: Secondary metabolites in plant defence mechanisms. *New Phytologist*, **127**, 617-633.

BENTLEY BL (1976) Plants bearing extrafloral nectaries and the associated ant community: Interhabitat differences in the reduction of herbivore damage. *Ecology*, **57**, 815-820.

BENTLEY BL (1977a) Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*, **8**, 407-427.

BENTLEY BL (1977b) The protective function of ants visiting the extrafloral nectaries of *Bixa orellana* (Bixaceae). *Journal of Ecology*, **65**, 27-38.

BERNASCONI ML, TURLINGS TCJ, AMBROSETTI L, BASSETTI P & DORN S (1998) Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomologia Experimentalis et Applicata*, **87**, 133-142.

BEZEMER TM, WAGENAAR R, VAN DAM NM, VAN DER PUTTEN WH & WÄCKERS FL (2004) Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. *Journal of Chemical Ecology*, **30**, 53-67.

BIRKENMEIER GF & RYAN CA (1998) Wound signaling in tomato plants - Evidence that ABA is not a primary signal for defense gene activation. *Plant Physiology*, **117**, 687-693.

BIRKETT MA, CAMPBELL CAM, CHAMBERLAIN K, GUERRIERI E, HICK AJ, MARTIN JL, MATTHES M, NAPIER JA, PETTERSSON J, PICKETT JA, POPPY GM, POW EM, PYE BJ, SMART LE, WADHAMS GH, WADHAMS LJ & WOODCOCK CM (2000) New roles for *cis*-jasmone as an insect semiochemical and in plant defense. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 9329-9334.

BLÜTHGEN N & FIEDLER K (2004) Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. *Journal of Animal Ecology*, **73**, 155-166.

BOECKLEN WJ (1984) The role of extrafloral nectaries in the herbivore defence of *Cassia fasciculata*. *Ecological Entomology*, **9**, 243-249.

BOHLMANN J, MARTIN D, OLDHAM NJ & GERSHENZON J (2000) Terpenoid secondary metabolism in *Arabidopsis thaliana*: cDNA cloning, characterization, and functional expression of a myrcene/(*E*)-β-ocimene synthase. *Archives of Biochemistry and Biophysics*, **375**, 261-269.

BOLAND W, FENG Z, DONATH J & GAEBLER A (1992) Are acyclic C_{11} and C_{16} homoterpenes plant volatiles indicating herbivory? *Naturwissenschaften*, **79**, 368-371.

BOLAND W, HOPKE J, DONATH J, NUSKE J & BUBLITZ F (1995) Jasmonic acid and coronatin induce odor production in plants. *Angewandte Chemie International Edition*, **34**, 1600-1602.

BOLTER CJ, DICKE M, VANLOON JJA, VISSER JH & POSTHUMUS MA (1997) Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *Journal of Chemical Ecology*, **23**, 1003-1023.

BOUWMEESTER HJ, VERSTAPPEN FWA, POSTHUMUS MA & DICKE M (1999) Spider mite-induced (3*S*)-(*E*)-nerolidol synthase activity in cucumber and Lima bean. The first dedicated step in acyclic C_{11}-homoterpene biosynthesis. *Plant Physiology*, **131**, 173-180.

BRAUDE EA, JACKMAN LM, LINSTEAD RP & LOWE G (1960) Hydrogen transfer. Part 13. The kinetics of dehydrogenation of 1,1-disubstituted 1,2-dihydronaphthalenes and tetralins by tetrachloro-1,2-benzoquinone. *Journal of the Chemical Society*, 3133-3138.

BRONSTEIN JL (1998) The contribution of ant-plant protection studies to our understanding of mutualism. *Biotropica*, **30**, 150-161.

BROUAT C, MCKEY D, BESSIERE JM, PASCAL L & HOSSAERT-MCKEY M (2000) Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: Preliminary results on *Leonardoxa* (Fabaceae: Caesalpinioideae) and *Petalomyrmex* (Formicidae: Formicinae). *Acta Oecologica*, **21**, 349-357.

BROWN RL, KAZAN K, MCGRATH KC, MACLEAN DJ & MANNERS JM (2003) A role for the GCC-box in jasmonate-mediated activation of the *PDF1.2* gene of *Arabidopsis*. *Plant Physiology*, **132**, 1020-1032.

BROWN WL (2000) Diversity of ants. Pages 45-79 in AGOSTI D, MAJER JD, ALONSO LE & SCHULTZ T (eds.) *Ants - Standard methods for measuring and monitoring biodiversity*. Smithonian Institution Press, Washington.

BRUCE TJA, MARTIN JL, PICKETT JA, PYE BJ, SMART LE & WADHAMS LJ (2003) *Cis*-Jasmone treatment induces resistance in wheat plants against the grain aphid, *Sitobion avenae* (Fabricius) (Homoptera: Aphididae). *Pest Management Science*, **59**, 1031-1036.

BRUIN J & DICKE M (2001) Chemical information transfer between wounded and unwounded plants: Backing up the future. *Biochemical Systematics and Ecology*, **29**, 1103-1113.

BRUIN J, DICKE M & SABELIS MW (1992) Plants are better protected against spidermites after exposure to volatiles from infested conspecifics. *Experientia*, **48**, 525-529.

BRUNKE EJ, HAMMERSCHMIDT FJ & SCHMAUS G (1993) Flower scent of some traditional medicinal plants. *ACS Symposium Series*, **525**, 282-296.

BUCKLEY RC (1982) Ant-plant interactions: a world review. Pages 111-162 in BUCKLEY RC (ed.) *Ant-plant interactions in Australia*. Junk, The Hague.

BUGG RL, ELLIS RT & CARLSON RW (1989) Ichneumonidae (Hymenoptera) using extrafloral nectar of Faba bean (*Vicia faba* L, Fabaceae) in Massachusetts. *Biological Agriculture & Horticulture*, **6**, 107-114.

BÚRQUEZ A & CORBET SA (1991) Do flowers reabsorb nectar? *Functional Ecology*, **5**, 369-379.

CHAMBERLAIN K, GUERRIERI E, PENNACCHIO F, PETTERSSON J, PICKETT JA, POPPY GM, POWELL W, WADHAMS LJ & WOODCOCK CM (2001) Can aphid-induced plant signals be transmitted aerially and through the rhizosphere? *Biochemical Systematics and Ecology*, **29**, 1063-1074.

CHEN F, THOLL D, D'AURIA JC, FAROOQ A, PICHERSKY E & GERSHENZON J (2003a) Biosynthesis and emission of ter-

penoid volatiles from *Arabidopsis* flowers. *Plant Cell*, **15**, 481-494.

CHEN F, D'AURIA JC, THOLL D, ROSS JR, GERSHENZON J, NOEL JP & PICHERSKY E (2003b) An *Arabidopsis thaliana* gene for methylsalicylate biosynthesis, identified by a biochemical genomics approach, has a role in defense. *Plant Journal*, **36**, 577-588.

CHEN F, RO DK, PETRI J, GERSHENZON J, BOHLMANN J, PICHERSKY E & THOLL D (2004) Characterization of a root-specific *Arabidopsis* terpene synthase responsible for the formation of the volatile monoterpene 1,8-cineole. *Plant Physiology*, **135**, 1956-1966.

CHENG SH, WILLMANN MR, CHEN HC & SHEEN J (2002) Calcium signaling through protein kinases. The *Arabidopsis* calcium-dependent protein kinase gene family. *Plant Physiology*, **129**, 469-485.

CIPOLLINI D, PURRINGTON CB & BERGELSON J (2003) Costs of induced responses in plants. *Basic and Applied Ecology*, **4**, 79-85.

CIPOLLINI D, ENRIGHT S, TRAW MB & BERGELSON J (2004) Salicylic acid inhibits jasmonic acid-induced resistance of *Arabidopsis thaliana* to *Spodoptera exigua*. *Molecular Ecology*, **13**, 1643-1653.

COGNI R, FREITAS AVL & OLIVEIRA PS (2003) Interhabitat differences in ant activity on plant foliage: Ants at extrafloral nectaries of *Hibiscus pernambucensis* in sandy and mangrove forests. *Entomologia Experimentalis et Applicata*, **107**, 125-131.

COLAZZA S, MCELFRESH JS & MILLAR JG (2004) Identification of volatile synomones, induced by *Nezara viridula* feeding and oviposition on bean spp, that attract the egg parasitoid *Trissolcus basalis*. *Journal of Chemical Ecology*, **30**, 945-964.

COUGHLIN RW (1969) Carbon as adsorbent and catalyst. *Industrial & Engineering Chemistry Product Research and Development*, **8**, 12-23.

CRANKSHAW DR & LANGENHEIM JH (1981) Variation in terpenes and phenolics through leaf development in *Hymenaea* and its possible significance to herbivory. *Biochemical Systematics and Ecology*, **9**, 115-124.

CREELMAN RA & MULLET JE (1997) Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 355-381.

CROFT KPC, JUTTNER F & SLUSARENKO AJ (1993) Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L) leaves inoculated with *Pseudomonas syringae* pv *Phaseolicola*. *Plant Physiology*, **101**, 13-24.

CUAUTLE M & RICO-GRAY V (2003) The effect of wasps and ants on the reproductive success of the extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). *Functional Ecology*, **17**, 417-423.

CURTIS JD & LERSTEN NR (1974) Morphology, seasonal variation, and function of resin glands on buds and leaves of *Populus deltoides* (Salicaceae). *American Journal of Botany*, **61**, 835-845.

D'AURIA JC, CHEN F & PICHERSKY E (2003) The SABATH family of MTs in *Arabidopsis thaliana* and other plant species. Pages 253-283 in ROMEO JT (ed.) *Recent advances in phytochemistry*. Elsevier Science Ltd, Oxford.

DAEHLER CC, YORKSTON M, SUN WG & DUDLEY N (1999) Genetic variation in morphology and growth characters of *Acacia koa* in the Hawaiian Islands. *International Journal of Plant Sciences*, **160**, 767-773.

DALY HV, DOYEN JT & PURCELL AH (1998) *Introduction to insect biology and diversity*. Oxford University Press, Oxford.

DAMMANN C, ROJO E & SANCHEZ-SERRANO JJ (1997) Abscisic acid and jasmonic acid activate wound-inducible genes in potato through separate, organ-specific signal transduction pathways. *Plant Journal*, **11**, 773-782.

DE BOER JG, POSTHUMUS MA & DICKE M (2004) Identification of volatiles that are used in discrimination between plants infested with prey or nonprey herbivores by a predatory mite. *Journal of Chemical Ecology*, **30**, 2215-2230.

DE MORAES CM, MESCHER MC & TUMLINSON JH (2001) Caterpillar-induced nocturnal plant volatiles repel nonspecific females. *Nature*, **410**, 577-580.

DE MORAES CM, LEWIS JM, PARÉ PW, ALBORN HT & TUMLINSON JH (1998) Herbivory-infested plants selectively attract parasitoids. *Nature*, **393**, 570-573.

DEBOUCK DG, MAQUET A & POESSO CE (1989) Biochemical evidence for two different gene pools in Lima bean, *Phaseolus lunatus* L. *Annual Report of the Bean Improvement Cooperation*, **32**, 58-59.

DEGENHARDT J & GERSHENZON J (2000) Demonstration and characterization of (*E*)-nerolidol synthase from maize: A herbivore-inducible terpene synthase participating in (3*E*)-4,8-dimethyl-1,3,7-nonatriene biosynthesis. *Planta*, **210**, 815-822.

DEGREEF J, ROCHA OJ, VANDERBORGHT T & BAUDOIN JP (2002) Soil seed bank and seed dormancy in wild populations of Lima bean (Fabaceae): Considerations for *in situ* and *ex situ* conservation. *American Journal of Botany*, **89**, 1644-1650.

DEJEAN A, MCKEY D, GIBERNAU M & BELIN M (2000) The arboreal ant mosaic in a Cameroonian rainforest (Hymenoptera: Formicidae). *Sociobiology*, **35**, 403-423.

DEVRIES PJ & BAKER I (1989) Butterfly exploitation of an ant-plant mutualism adding insult to herbivory. *Journal of the New York Entomological Society*, **97**, 332-340.

DEWITT TJ, SIH A & WILSON DS (1998) Costs and limits of phenotypic plasticity. *Trends in Ecology and Evolution*, **13**, 77-81.

DICKE M (1986) Volatile spider-mite pheromone and host-plant kairomone, involved in spaced-out gregariousness in the spider mite *Tetranychus urticae*. *Physiological Entomology*, **11**, 251-262.

DICKE M (1994) Local and systemic production of volatile herbivore-induced terpenoids: Their role in plant-carnivore mutualism. *Journal of Plant Physiology*, **143**, 465-472.

DICKE M (1999a) Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomologia Experimentalis et Applicata*, **91**, 131-142.

DICKE M (1999b) Specifity of herbivore-induced plant defences. Pages 43-54 in CHADWICK DJ & GOODE JA (eds.) *Insect-plant interactions and induced plant defence*. John Wiley & Sons Ltd, Chichester.

DICKE M (1999c) Evolution of induced indirect defences of plants. Pages 62-88 in TOLLRIAN R & HARVELL CD (eds.) *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton.

DICKE M & GROENEVELD A (1986) Hierarchical structure in kairomone preference on the predatory mite *Amblyseius potentillae*: Dietary component indispensable for diapause induction affects prey location behaviour. *Ecological Entomology*, **11**, 131-138.

DICKE M & SABELIS MW (1989) Does it pay plants to advertize for bodyguards? Towards a cost-benefit analysis of induced synomone production. Pages 341-358 in LAMBERS H, CAMBRIDGE ML,

KONINGS H & PONS TL (eds.) *Variation in growth rate and productivity of higher plants*. SPB Academic Publishing, The Hague.

DICKE M & VET LEM (1999) Plant-carnivore interactions: Evolutionary and ecological consequences for plant, herbivore and carnivore. Pages 483-520 in OLFF H, BROWN VK & DRENT RH (eds.) *Herbivores: Between plants and predators*. Blackwell Science, Oxford.

DICKE M & VAN LOON JJA (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata*, **97**, 237-249.

DICKE M & DIJKMAN H (2001) Within-plant circulation of systemic elicitor of induced defence and release from roots of elicitor that affects neighbouring plants. *Biochemical Systematics and Ecology*, **29**, 1075-1087.

DICKE M, AGRAWAL AA & BRUIN J (2003a) Plants talk, but are they deaf? *Trends in Plant Science*, **8**, 403-405.

DICKE M, VAN DER MAAS J, TAKABAYASHI J & VET LEM (1990a) Learning affects response to volatile allelochemicals by predatory mites. *Proceedings of Experimental and Applied Entomology*, **1**, 31-36.

DICKE M, VANBAARLEN P, WESSELS R & DIJKMAN H (1993) Herbivory induces systemic production of plant volatiles that attract predators of the herbivore: Extraction of endogenous elicitor. *Journal of Chemical Ecology*, **19**, 581-599.

DICKE M, GOLS R, LUDEKING D & POSTHUMUS MA (1999) Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in Lima bean plants. *Journal of Chemical Ecology*, **25**, 1907-1922.

DICKE M, DE BOER JG, HOFTE M & ROCHA-GRANADOS MC (2003b) Mixed blends of herbivore-induced plant volatiles and foraging success of carnivorous arthropods. *Oikos*, **101**, 38-48.

DICKE M, SABELIS MW, TAKABAYASHI J, BRUIN J & POSTHUMUS MA (1990b) Plant strategies of manipulating predator-prey interactions through allelochemicals: Prospects for application in pest control. *Journal of Chemical Ecology*, **16**, 3091-3118.

DICKE M, VANBEEK TA, POSTHUMUS MA, BENDOM N, VANBOKHOVEN H & DE-GROOT AE (1990c) Isolation and identification of volatile kairomone that affects acarine predator-prey interactions - Involvement of host plant in its production. *Journal of Chemical Ecology*, **16**, 381-396.

DISNEY RHL (1994) *Scuttle flies: The Phoridae*. Chapman & Hall, London.

DJIETO-LORDON C & DEJEAN A (1999a) Tropical arboreal ant mosaics: Innate attraction and imprinting determine nest site selection in dominant ants. *Behavioral Ecology and Sociobiology*, **45**, 219-225.

DJIETO-LORDON C & DEJEAN A (1999b) Innate attraction supplants experience during host selection in an obligate plant-ant. *Behavioural Processes*, **46**, 181-187.

DOLCH R & TSCHARNTKE T (2000) Defoliation of alders (*Alnus glutinosa*) affects herbivory by leaf beetles on undamaged neighbours. *Oecologia*, **125**, 504-511.

DONATH J & BOLAND W (1995) Biosynthesis of acyclic homoterpenes: Enzyme selectivity and absolute configuration of the nerolidol precursor. *Phytochemistry*, **39**, 785-790.

DREISIG H (2000) Defense by exploitation in the Florida carpenter ant, *Camponotus floridanus*, at an extrafloral nectar resource. *Behavioral Ecology and Sociobiology*, **47**, 274-279.

DRUKKER B, SCUTAREANU P & SABELIS MW (1995) Do anthocorid predators respond to synomones from *Psylla*-infested

pear trees under field conditions. *Entomologia Experimentalis et Applicata*, **77**, 193-203.

DU YJ, POPPY GM & POWELL W (1996) Relative importance of semiochemicals from first and second trophic levels in host foraging behavior of *Aphidius ervi*. *Journal of Chemical Ecology*, **22**, 1591-1605.

DU YJ, POPPY GM, POWELL W, PICKETT JA, WADHAMS LJ & WOODCOCK CM (1998) Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *Journal of Chemical Ecology*, **24**, 1355-1368.

DUDAREVA N, MARTIN D, KISH CM, KOLOSOVA N, GORENSTEIN N, FÄLDT J, MILLER B & BOHLMANN J (2003) (*E*)-β-Ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: Function and expression of three terpene synthase genes of a new terpene synthase subfamily. *Plant Cell*, **15**, 1227-1241.

DUFFEY SS (1980) Sequestration of plant natural products by insects. *Annual Review of Entomology*, **25**, 447-477.

DUFFEY SS & STOUT MJ (1996) Antinutritive and toxic components of plant defence against insects. *Archives of Insect Biochemistry and Physiology*, **32**, 3-37.

DUSSOURD DE & EISNER T (1987) Vein-cutting behavior insect counterploy to the latex defense of plants. *Science*, **237**, 898-901.

DUSSOURD DE & DENNO RF (1991) Deactivation of plant defense - Correspondence between insect behavior and secretory canal architecture. *Ecology*, **72**, 1383-1396.

EHRLICH PR & RAVEN PH (1964) Butterflies and plants: A study in coevolution. *Evolution*, **18**, 586-608.

ELIAS TS (1972) Morphology and anatomy of foliar nectaries of *Pithecellobium macradenium* (Leguminosae). *Botanical Gazette*, **133**, 38-42.

ELIAS TS (1983) Extrafloral nectaries: Their structure and distribution. Pages 174-203 in BENTLEY B & ELIAS TS (eds.) *The biology of nectaries*. Columbia University Press, New York.

ENGELBERTH J, ALBORN HT, SCHMELZ EA & TUMLINSON JH (2004) Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 1781-1785.

ENGELBERTH J, KOCH T, SCHÜLER G, BACHMANN N, RECHTENBACH J & BOLAND W (2001) Ion channel-forming alamethicin is a potent elicitor of volatile biosynthesis and tendril coiling. Cross talk between jasmonate and salicylate signaling in Lima bean. *Plant Physiology*, **125**, 369-377.

ENGLISH LOEB GM & KARBAN R (1991) Consequences of mite feeding injury to beans on the fecundity and survivorship of the two-spotted spider mite (Acari: Tetranychidae). *Experimental & Applied Acarology*, **11**, 125-136.

FAHN A (1988) Secretory tissues in vascular plants. *New Phytologist*, **108**, 229-257.

FAHN A (2000) Structure and function of secretory cells. *Advances in Botanical Research*, **31**, 37-75.

FÄLDT J, ARIMURA G, GERSHENZON J, TAKABAYASHI J & BOHLMANN J (2003) Functional identification of *AtTPS03* as (*E*)-β-ocimene synthase: A monoterpene synthase catalyzing jasmonate- and wound-induced volatile formation in *Arabidopsis thaliana*. *Planta*, **216**, 745-751.

FARAG MA & PARÉ PW (2002) C_6-Green leaf volatiles trigger local and systemic VOC emissions in tomato. *Phytochemistry*, **61**, 545-554.

FARMER EE (2001) Surface-to-air signals. *Nature*, **411**, 854-856.

FARMER EE & RYAN CA (1990) Interplant communication - Airborne methyl jasmonate induces synthesis of proteinase-inhibitors in plant-leaves. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 7713-7716.

FELTON GW & EICHENSEER H (1999) Herbivore saliva and its effects on plant defense against herbivores and pathogens. Pages 19-36 in AGRAWAL AA, TUZUN S & BENT E (eds.) *Induced plant defenses against pathogens and herbivores: Ecology and agriculture*. American Phytopathology Society Press, St. Paul.

FIALA B & MASCHWITZ U (1990) Studies on the south east asian ant-plant association *Crematogaster borneensis*/*Macaranga*: Adaptations of the ant partner. *Insectes Sociaux*, **37**, 212-231.

FIGIER J (1971) Fine structure in extrafloral nectary of *Vicia faba* L. *Planta*, **98**, 31-49.

FOFANA B, DU JARDIN P & BAUDOIN JP (2001) Genetic diversity in the Lima bean (*Phaseolus lunatus* L) as revealed by chloroplast DNA (cpDNA) variations. *Genetic Resources and Crop Evolution*, **48**, 437-445.

FOFANA B, VEKEMANS X, DU JARDIN P & BAUDOIN JP (1997) Genetic diversity in Lima bean (*Phaseolus lunatus* L) as revealed by RAPD markers. *Euphytica*, **95**, 157–165.

FOSTER WA (1995) Mosquito sugar feeding and reproductive energetics. *Annual Review of Entomology*, **40**, 443-474.

FOWLER SV & MACGARVIN M (1985) The impact of hairy wood ants *Formica lugubris* on the guild structure of herbivorous insects on birch, *Betula pubescens*. *Journal of Animal Ecology*, **54**, 847-856.

FOWLER SV & LAWTON JH (1985) Rapidly induced defenses and talking trees: The devil's advocate position. *American Naturalist*, **126**, 181-195.

FREITAS L, GALETTO L, BERNARDELLO G & PAOLI AAS (2000) Ant exclusion and reproduction of *Croton sarcopetalus* (Euphorbiaceae). *Flora*, **195**, 398-402.

FREY M, SPITELLER D, BOLAND W & GIERL A (2004) Transcriptional activation of *Igl*, the gene for indole formation in *Zea mays*: A structure-activity study with elicitor-active *N*-acyl glutamines from insects. *Phytochemistry*, **65**, 1047-1055.

FREY M, STETTNER C, PARÉ PW, SCHMELZ EA, TUMLINSON JH & GIERL A (2000) An herbivore elicitor activates the gene for indole emission in maize. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 14801-14806.

FREY-WYSSLING A (1955) The phloem supply to nectaries. *Acta Botanica Neerlandica*, **4**, 358-369.

FRITSCHE HOBALLAH ME & TURLINGS TCJ (2001) Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evolutionary Ecology Research*, **3**, 553-565.

FRY JD (1989) Evolutionary adaptation to host plants in a laboratory population of the phytophagous mite *Tetranychus urticae* Koch. *Oecologia*, **81**, 559-565.

FUJIMOTO SY, OHTA M, USUI A, SHINSHI H & OHME-TAKAGI M (2000) *Arabidopsis* ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. *Plant Cell*, **12**, 393-404.

FUNK CJ (2001) Alkaline phosphatase activity in whitefly salivary glands and saliva. *Archives of Insect Biochemistry and Physiology*, **46**, 165-174.

GALETTO L & BERNARDELLO LM (1992) Extrafloral nectaries that attract ants in Bromeliaceae: Structure and nectar com-

position. *Canadian Journal of Botany*, **70**, 1101-1106.

GALIZIA CG, SACHSE S & MUSTAPARTA H (2000) Calcium responses to pheromones and plant odours in the antennal lobe of the male and female moth *Heliothis virescens*. *Journal of Comparative Physiology A – Sensory, Neural, and Behavioral Physiology*, **186**, 1049-1063.

GANG DR, WANG JH, DUDAREVA N, NAM KH, SIMON JE, LEWINSOHN E & PICHERSKY E (2001) An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. *Plant Physiology*, **125**, 539-555.

GARDNER SN & AGRAWAL AA (2002) Induced plant defence and the evolution of counter-defences in herbivores. *Evolutionary Ecology Research*, **4**, 1131-1151.

GARY RE & FOSTER WA (2004) *Anopheles gambiae* feeding and survival on honeydew and extrafloral nectar of peridomestic plants. *Medical and Veterinary Entomology*, **18**, 102-107.

GENTRY G (2003) Multiple parasitoid visitors to the extrafloral nectaries of *Solanum adherens*. Is *S. adherens* an insectary plant? *Basic and Applied Ecology*, **4**, 405-411.

GERSHENZON J (1994a) Metabolic costs of terpenoid accumulation in higher plants. *Journal of Chemical Ecology*, **20**, 1281-1328.

GERSHENZON J (1994b) The cost of plant chemical defense against herbivores: A biochemical perspective. Pages 105-173 in BERNAYS EA (ed.) *Insect-plant interactions*. CRC Press, Boca Raton.

GETTY T (1996) The maintenance of phenotypic plasticity as a signal detection problem. *American Naturalist*, **148**, 378-385.

GILL FB (1988) Effects of nectar removal on nectar accumulation in flowers of *Heliconia imbricata* (Heliconiaceae). *Biotropica*, **20**, 169-171.

GNANVOSSOU D, HANNA R, DICKE M & YANINEK SJ (2001) Attraction of the predatory mites *Typhlodromalus manihoti* and *Typhlodromalus aripo* to cassava plants infested by cassava green mite. *Entomologia Experimentalis et Applicata*, **101**, 291-298.

GODFRAY HCJ (1995) Communication between the first and 3rd trophic levels - An analysis using biological signalling theory. *Oikos*, **72**, 367-374.

GOLS R, ROOSJEN M, DIJKMAN H & DICKE M (2003) Induction of direct and indirect plant responses by jasmonic acid, low spider mite densities, or a combination of jasmonic acid treatment and spider mite infestation. *Journal of Chemical Ecology*, **29**, 2651-2666.

GOMI K, YAMASAKI Y, YAMAMOTO H & AKIMITSU K (2003) Characterization of a hydroperoxide lyase gene and effect of C_6-volatiles on expression of genes of the oxylipin metabolism in *Citrus*. *Journal of Plant Physiology*, **160**, 1219-1131.

GOUINGUENE S, DEGEN T & TURLINGS TCJ (2001) Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology*, **11**, 9-16.

GOUINGUENE S, ALBORN H & TURLINGS TCJ (2003) Induction of volatile emissions in maize by different larval instars of *Spodoptera littoralis*. *Journal of Chemical Ecology*, **29**, 145-162.

GULMON SL & MOONEY HA (1986) Costs of defense and their effects on plant productivity. Pages 151-197 in GIVNISH TJ (ed.) *On the economy of plant form and function*. Cambridge University Press, Cambridge.

GUTIÉRREZ-SALGADO A, GEPTS P & DEBOUCK DG (1995) Evidence for two gene pools of the Lima bean, *Phaseolus lunatus* L, in the Americas. *Genetic Resources and Crop Evolution*, **42**, 15-28.

HALITSCHKE R & BALDWIN IT (2004) Jasmonates and related compounds in plant-insect interactions. *Journal of Plant Growth Regulation*, **23**, 238-245.

HALITSCHKE R, SCHITTKO U, POHNERT G, BOLAND W & BALDWIN IT (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. *Plant Physiology*, **125**, 711-717.

HALITSCHKE R, GASE K, HUI DQ, SCHMIDT DD & BALDWIN IT (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VI. Microarray analysis reveals that most herbivore-specific transcriptional changes are mediated by fatty acid-amino acid conjugates. *Plant Physiology*, **131**, 1894-1902.

HAMILTON-KEMP TR, LOUGHRIN JH, ARCHBOLD DD, ANDERSEN RA & HILDEBRAND DF (1991) Inhibition of pollen germination by volatile compounds including 2-hexenal and 3-hexenal. *Journal of Agricultural and Food Chemistry*, **39**, 952-956.

HARBORNE JB (1988) *Introduction to ecological biochemistry*. Academic Press, London.

HARDING J & TUCKER CL (1969) Quantitative studies on mating systems: III. Methods for the estimation of male gametophytic selective values and differential outcrossing rates. *Evolution*, **23**, 85-95.

HARMON AC, GRIBSKOV M & HARPER JF (2000) CDPKs - A kinase for every Ca^{2+} signal? *Trends in Plant Science*, **5**, 154-159.

HARMS K, ATZORN R, BRASH A, KUHN H, WASTERNACK C, WILLMITZER L & PENA-CORTÉS H (1995) Expression of a flax allene oxide synthase cDNA leads to increased endogenous jasmonic acid (JA) levels in transgenic potato plants but not to a corresponding activation of JA-responding genes. *Plant Cell*, **7**, 1645-1654.

HARRISON JM & BREED MD (1987) Temporal learning in the giant tropical ant *Paraponera clavata*. *Physiological Entomology*, **12**, 317-320.

HARVEY JA (2000) Dynamic effects of parasitism by an endoparasitoid wasp on the development of two host species: Implications for host quality and parasitoid fitness. *Ecological Entomology*, **25**, 267-278.

HATANAKA A (1993) The biogeneration of green odor by green leaves. *Phytochemistry*, **34**, 1201-1218.

HAUKIOJA E & HAKALA T (1975) Herbivore cycles and periodic outbreaks: Formulation of a general hypothesis. *Reports from the Kevo Subarctic Research Station*, **12**, 1-9.

HEADS PA & LAWTON JH (1985) Bracken ants and extrafloral nectaries 3. How insect herbivores avoid ant predation. *Ecological Entomology*, **10**, 29-42.

HEIL M (2001) The ecological concept of costs of induced systemic resistance (ISR). *European Journal of Plant Pathology*, **107**, 137-146.

HEIL M (2002) Ecological costs of induced resistance. *Current Opinion in Plant Biology*, **5**, 345-350.

HEIL M (2004a) Induction of two indirect defences benefits Lima bean (*Phaseolus lunatus*, Fabaceae) in nature. *Journal of Ecology*, **92**, 527-536.

HEIL M (2004b) Direct defense or ecological costs: Responses of herbivorous beetles to volatiles released by wild Lima bean. *Journal of Chemical Ecology*, **30**, 1289-1295.

HEIL M & BALDWIN IT (2002) Fitness costs of induced resistance: Emerging experimental support for a slippery concept. *Trends in Plant Science*, **7**, 61-67.

HEIL M & MCKEY D (2003) Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 425-453.

HEIL M, STAEHELIN C & MCKEY D (2000a) Low chitinase activity in *Acacia* myrmecophytes: A potential trade-off between biotic and chemical defences? *Naturwissenschaften*, **87**, 555-558.

HEIL M, RATTKE J & BOLAND W (2005) Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science*, **308**, 560-563.

HEIL M, FIALA B, BOLLER T & LINSENMAIR KE (1999) Reduced chitinase activities in ant plants of the genus *Macaranga*. *Naturwissenschaften*, **86**, 146-149.

HEIL M, FIALA B, BAUMANN B & LINSENMAIR KE (2000b) Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Functional Ecology*, **14**, 749-757.

HEIL M, HILPERT A, KRÜGER R & LINSENMAIR KE (2004a) Competition among visitors to extrafloral nectaries as a source of ecological costs of an indirect defence. *Journal of Tropical Ecology*, **20**, 201-208.

HEIL M, KOCH T, HILPERT A, FIALA B, BOLAND W & LINSENMAIR KE (2001) Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius,* is an induced, indirect, defensive response elicited by jasmonic acid. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 1083-1088.

HEIL M, DELSINNE T, HILPERT A, SCHURKENS S, ANDARY C, LINSENMAIR KE, SOUSA MS & MCKEY D (2002) Reduced chemical defence in ant-plants? A critical re-evaluation of a widely accepted hypothesis. *Oikos*, **99**, 457-468.

HEIL M, GREINER S, MEIMBERG H, KRÜGER R, NOYER JL, HEUBL G, LINSENMAIR KE & BOLAND W (2004b) Evolutionary change from induced to constitutive expression of an indirect plant resistance. *Nature*, **430**, 205-208.

HELDER RJ (1958) The excretion of carbohydrates (nectaries). Pages 978-990 in RUHLAND W (ed.) *Handbuch der Pflanzenphysiologie*. Springer-Verlag, Berlin.

HERMS DA & MATTSON WJ (1992) The dilemma of plants: To grow or to defend. *The Quarterly Review of Biology*, **67**, 283-335.

HILDEBRAND DF, BROWN GC, JACKSON DM & HAMILTON-KEMP TR (1993) Effects of some leaf-emitted volatile compounds on aphid population increase. *Journal of Chemical Ecology*, **19**, 1875-1887.

HILKER M & MEINERS T (2002) Induction of plant responses to oviposition and feeding by herbivorous arthropods: A comparison. *Entomologia Experimentalis et Applicata*, **104**, 181-192.

HOBALLAH ME, KÖLLNER TG, DEGENHARDT J & TURLINGS TCJ (2004) Costs of induced volatile production in maize. *Oikos*, **105**, 168-180.

HÖLLDOBLER B & LUMSDEN CJ (1980) Territorial strategies in ants. *Science*, **210**, 732-739.

HÖLLDOBLER B & WILSON EO (1990) *The ants*. Springer-Verlag, Berlin.

HÖLLER C, MICHA SG, SCHULZ S, FRANCKE W & PICKETT JA (1994) Enemy-induced dispersal in a parasitic wasp. *Experientia*, **50**, 182-185.

HONOMICHL K, BELLMANN H, JACOBS W & RENNER M (1996) *Biologie und Ökologie der Insekten*. Gustav Fischer Verlag, Stuttgart.

HOPKE J, DONATH J, BLECHERT S & BOLAND W (1994) Herbivore-induced volatiles - The emission of acyclic homoterpenes from leaves of *Phaseolus lunatus* and *Zea mays* can be triggered by a β-glucosidase and jasmonic acid. *FEBS Letters*, **352**, 146-150.

HORIUCHI J, ARIMURA G, OZAWA R, SHIMODA T, TAKABAYASHI J & NISHIOKA T (2001) Exogenous ACC enhances volatiles production mediated by jasmonic acid in Lima bean leaves. *FEBS Letters*, **509**, 332-336.

HORIUCHI J, ARIMURA G, OZAWA R, SHIMODA T, TAKABAYASHI J & NISHIOKA T (2003a) A comparison of the responses of *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae) to volatiles emitted from Lima bean leaves with different levels of damage made by *T. urticae* or *Spodoptera exigua* (Lepidoptera: Noctuidae). *Applied Entomology and Zoology*, **38**, 109-116.

HORIUCHI JI, ARIMURA GI, OZAWA R, SHIMODA T, DICKE M, TAKABAYASHI J & NISHIOKA T (2003b) Lima bean leaves exposed to herbivore-induced conspecific plant volatiles attract herbivores in addition to carnivores. *Applied Entomology and Zoology*, **38**, 365-368.

HORVITZ CC & SCHEMSKE DW (1984) Effects of ants and an ant-tended herbivore on seed production of a neotropical herb. *Ecology*, **65**, 1369-1378.

HUNTER MD (2002) A breath of fresh air: Beyond laboratory studies of plant volatile-natural enemy interactions. *Agricultural and Forest Entomology*, **4**, 81-86.

INOUYE DW & TAYLOR ORJ (1979) A temperate region plant ant seed predator system: Consequences of extra floral nectar secretion by *Helianthella quinquenervis*. *Ecology*, **60**, 1-7.

INUI Y, MIYAMOTO Y & OHGUSHI T (2003) Comparison of volatile leaf compounds and herbivorous insect communities on three willow species. *Population Ecology*, **45**, 41-46.

JACOB HS & EVANS EW (1998) Effects of sugar spray and aphid honeydew on field populations of the parasitoid *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae). *Environmental Entomology*, **27**, 1563-1568.

JAMES DG (2005) Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *Journal of Chemical Ecology*, **31**, 481-495.

JANSSEN A, SABELIS MW & BRUIN J (2002) Evolution of herbivore-induced plant volatiles. *Oikos*, **97**, 134-138.

JANSSEN A, VANALPHEN JJM, SABELIS MW & BAKKER K (1995a) Odor-mediated avoidance of competition in *Drosophila* parasitoids: The ghost of competition. *Oikos*, **73**, 356-366.

JANSSEN A, VANALPHEN JJM, SABELIS MW & BAKKER K (1995b) Specificity of odor mediated avoidance of competition in *Drosophila* parasitoids. *Behavioral Ecology and Sociobiology*, **36**, 229-235.

JANSSEN A, BRUIN J, JACOBS G, SCHRAAG R & SABELIS MW (1997) Predators use volatiles to avoid prey patches with conspecifics. *Journal of Animal Ecology*, **66**, 223-232.

JANZEN DH (1966) Coevolution of mutualism between ants and acacias in Central America. *Evolution*, **20**, 249-275.

JÄREMO J, TUOMI J & NILSSON P (1999) Adaptive status of localized and systemic defense responses in plants. Pages 31-44 in TOLLIAN R & HARVELL CD (eds.) *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton.

JONES CG, HOPPER RF, COLEMAN JS & KRISCHIK VA (1993) Control of systemi-

cally induced herbivore resistance by plant vascular architecture. *Oecologia*, **93**, 452-456.

KAHL J, SIEMENS DH, AERTS RJ, GABLER R, KÜHNEMANN F, PRESTON CA & BALDWIN IT (2000) Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. *Planta*, **210**, 336-342.

KAISER R & LAMPARSKY D (1977) Nouveaux constituants de l'absolue de jacinthe et leur comportment olfactif. *Parfums, Cosmétiques, Arômes*, **17**, 71-79.

KALBERER NM, TURLINGS TCJ & RAHIER M (2001) Attraction of a leaf beetle (*Oreina cacaliae*) to damaged host plants. *Journal of Chemical Ecology*, **27**, 647-661.

KARBAN R (1989) Fine-scale adaptation of herbivorous thrips to individual host plants. *Nature*, **340**, 60-61.

KARBAN R & BALDWIN IT (1997) *Induced responses to herbivory*. University of Chicago Press, Chicago.

KARBAN R & MARON J (2002) The fitness consequences of interspecific eavesdropping between plants. *Ecology*, **83**, 1209-1213.

KARBAN R, AGRAWAL AA, THALER JS & ADLER LS (1999) Induced plant responses and information content about risk of herbivory. *Trends in Ecology and Evolution*, **14**, 443-447.

KARBAN R, BALDWIN I, BAXTER K, LAUE G & FELTON G (2000) Communication between plants: Induced resistance in wild tobacco plants following clipping of neighboring sagebrush. *Oecologia*, **125**, 66-71.

KEELER KH (1979) Species with extrafloral nectaries in a temperate flora (Nebraska). *Prairie Naturalist*, **11**, 33-38.

KEELING CI, PLETTNER E & SLESSOR KN (2004) Hymenopteran semiochemicals. *Topics in Current Chemistry*, **239**, 133-177.

KELSEY LP (1969) A revision of the Scenopinidae (Diptera) of the world. *Bulletin of the United States National Museum*, **277**, 1-336.

KELSEY LP (1981) New Scenopinidae (Diptera) from the Palearctic. *Folia Entomologica Hungarica*, **2**, 85-93.

KESSLER A & BALDWIN I (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, **291**, 2141-2144.

KESSLER A, HALITSCHKE R & BALDWIN IT (2004) Silencing the jasmonate cascade: Induced plant defenses and insect populations. *Science*, **305**, 665-668.

KIM CY, LIU YD, THORNE ET, YANG HP, FUKUSHIGE H, GASSMANN W, HILDEBRAND D, SHARP RE & ZHANG SQ (2003) Activation of a stress-responsive mitogen-activated protein kinase cascade induces the biosynthesis of ethylene in plants. *Plant Cell*, **15**, 2707-2718.

KISHIMOTO K, MATSUI K, OZAWA R & TAKABAYASHI J (2005) Volatile C_6-aldehydes and allo-ocimene activate defense genes and induce resistance against *Botrytis cinerea* in *Arabidopsis thaliana*. *Plant and Cell Physiology*, **46**, 1093-1102.

KOBAYASHI Y & YAMAMURA N (2003) Evolution of signal emission by non-infested plants growing near infested plants to avoid future risk. *Journal of Theoretical Biology*, **223**, 489-503.

KOCH T (2001). *Analytik pflanzlicher Signalstoffe: Phytohormone und Abwehr*. PhD thesis, Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena.

KOCH T, KRUMM T, JUNG V, ENGELBERTH J & BOLAND W (1999) Differential induction of plant volatile biosynthesis in the

Lima bean by early and late intermediates of the octadecanoid-signaling pathway. *Plant Physiology*, **121**, 153-162.

KODAMA H, NISHIUCHI T, SEO S, OHASHI Y & IBA K (2000) Possible involvement of protein phosphorylation in the wound-responsive expression of *Arabidopsis* plastid ω-3 fatty acid desaturase gene. *Plant Science*, **155**, 153-160.

KOPTUR S (1983) Flowering phenology and floral biology of *Inga* (Fabaceae: Mimosoideae). *Systematic Botany*, **8**, 354-368.

KOPTUR S (1985) Alternative defenses against herbivores in *Inga* (Fabaceae, Mimosoideae) over an elevational gradient. *Ecology*, **66**, 1639-1650.

KOPTUR S (1992) Extrafloral nectary-mediated interactions between insects and plants. Pages 81-129 in BERNAYS E (ed.) *Insect-plant interactions*. CRC Press, Boca Raton.

KOPTUR S & LAWTON JH (1988) Interactions among vetches bearing extrafloral nectaries their biotic protective agents and herbivores. *Ecology*, **69**, 278-283.

KOST C & HEIL M (2005a) Increased availability of extrafloral nectar reduces herbivory in Lima beans (*Phaseolus lunatus*, Fabaceae). *Basic and Applied Ecology*, **6**, 237-248.

KOST C & HEIL M (2005b) Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. *Journal of Ecology*, accepted.

KRIPS OE, WILLEMS PEL, GOLS R, POST-HUMUS MA, GORT G & DICKE M (2001) Comparison of cultivars of ornamental crop *Gerbera jamesonii* on production of spider mite-induced volatiles, and their attractiveness to the predator *Phytoseiulus persimilis*. *Journal of Chemical Ecology*, **27**, 1355-1372.

KRUMM T, BANDEMER K & BOLAND W (1995) Induction of volatile biosynthesis in the Lima bean (*Phaseolus lunatus*) by leucine- and isoleucine conjugates of 1-oxo- and 1-hydroxyindan-4-carboxylic acid: Evidence for amino acid conjugates of jasmonic acid as intermediates in the octadecanoid signalling pathway. *FEBS Letters*, **377**, 523-529.

KUNERT M, BIEDERMANN A, KOCH T & BOLAND W (2002) Ultrafast sampling and analysis of plant volatiles by a hand-held miniaturised GC with pre-concentration unit: Kinetic and quantitative aspects of plant volatile production. *Journal of Separation Science*, **25**, 677-684.

KUO J & PATE JS (1985) The extrafloral nectaries of cowpea (*Vigna unguiculata* (L) Walp): I. Morphology, anatomy and fine structure. *Planta*, **166**, 15-27.

LABEYRIE E, PASCAL L, DELABIE J, ORIVEL J, DEJEAN A & HOSSAERT-MCKEY M (2001) Protection of *Passiflora glandulosa* (Passifloraceae) against herbivory: Impact of ants exploiting extrafloral nectaries. *Sociobiology*, **38**, 317-321.

LANDOLT PJ (1993) Effects of host plant leaf damage on cabbage looper moth attraction and oviposition. *Entomologia Experimentalis et Applicata*, **67**, 79-85.

LAUDERT D & WEILER EW (1998) Allene oxide synthase: A major control point in *Arabidopsis thaliana* octadecanoid signalling. *Plant Journal*, **15**, 675-684.

LEE GI & HOWE GA (2003) The tomato mutant *spr1* is defective in systemin perception and the production of a systemic wound signal for defense gene expression. *Plant Journal*, **33**, 567-576.

LEITNER M, BOLAND W & MITHÖFER A (2005) Direct and indirect defences induced by piercing-sucking and chewing herbivores in *Medicago truncatula*. *New Phytologist*, **167**, 597-606.

LEÓN J, ROJO E & SÁNCHEZ-SERRANO JJ (2001) Wound signalling in plants. *Journal of Experimental Botany*, **52**, 1-9.

LI L, LI CY, LEE GI & HOWE GA (2002) Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 6416-6421.

LICHTENTHALER HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 47-65.

LIU YD, JIN HL, YANG KY, KIM CY, BAKER B & ZHANG SQ (2003) Interaction between two mitogen-activated protein kinases during tobacco defense signaling. *Plant Journal*, **34**, 149-160.

LOEFFLER C, BERGER S, GUY A, DURAND T, BRINGMANN G, DREYER M, VON RAD U, DURNER J & MUELLER MJ (2005) B_1-phytoprostanes trigger plant defense and detoxification responses. *Plant Physiology*, **137**, 328-340.

LOUGHRIN JH, MANUKIAN A, HEATH RR & TUMLINSON JH (1995) Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *Journal of Chemical Ecology*, **21**, 1217-1227.

LOUGHRIN JH, MANUKIAN A, HEATH RR, TURLINGS TCJ & TUMLINSON JH (1994) Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 11836-11840.

LÜCKER J, BOUWMEESTER HJ, SCHWAB W, BLAAS J, VAN DER PLAS LHW & VERHOEVEN HA (2001) Expression of *Clarkia S*-linalool synthase in transgenic petunia plants results in the accumulation of *S*-linalyl-β-D-glucopyranoside. *Plant Journal*, **27**, 315-324.

LUDWIG AA, ROMEIS T & JONES JDG (2004) CDPK-mediated signalling pathways: Specificity and cross-talk. *Journal of Experimental Botany*, **55**, 181-188.

LUNAU K (2004) Adaptive radiation and coevolution - Pollination biology case studies. *Organisms Diversity & Evolution*, **4**, 207-224.

LÜTTGE U (1971) Structure and function of plant glands. *Annual Review of Plant Physiology*, **22**, 23-44.

MACKAY DA & WHALEN MA (1998) Associations between ants (Hymenoptera: Formicidae) and *Adriana* Gaudich. (Euphorbiaceae) in East Gippsland. *Australian Journal of Entomology*, **37**, 335-339.

MAFFEI M, BOSSI S, SPITELLER D, MITHÖFER A & BOLAND W (2004) Effects of feeding *Spodoptera littoralis* on Lima bean leaves: I. Membrane potentials, intracellular calcium variations, oral secretions, and regurgitate components. *Plant Physiology*, **134**, 1752-1762.

MAKINO B, KAWAI M, YAMAMOTO T, YAMAMURA H, BUTSUGAN Y, HAYASHI M & OGAWA K (1992) Activated charcoal-mediated hydroxylation under very mild conditions - Conversion of physalin-B into 25-hydroxyphysalin-B. *Journal of the Chemical Society-Chemical Communications*, 1430-1431.

MALDONADO AM, DOERNER P, DIXON RA, LAMB CJ & CAMERON RK (2002) A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis*. *Nature*, **419**, 399-403.

MALECK K & DIETRICH RA (1999) Defense on multiple fronts: How do plants cope with diverse enemies? *Trends in Plant Science*, **4**, 215-219.

MAQUET A, GUTIERREZ A & DEBOUCK DG (1990) Further biochemical evidence for the existence of two gene pools in Lima beans. *Annual Reports of the Bean Improvement Cooperation*, **33**, 128-129.

MATILE L (1997) Phylogeny and evolution of the larval diet in the Sciaroidea (Diptera,

Bibionomorpha) since the Mesozoic. *Memoires du Museum National d'Histoire Naturelle*, **173**, 273-303.

MATSUI K, KURISHITA S, HISAMITSU A & KAJIWARA T (2000) A lipid-hydrolysing activity involved in hexenal formation. *Biochemical Society Transactions*, **28**, 857-860.

MATTIACCI L, DICKE M & POSTHUMUS MA (1994) Induction of parasitoid attracting synomone in Brussel sprouts plants by feeding *Pieris brassicae* larvae: Role of mechanical damage and herbivore elicitor. *Journal of Chemical Ecology*, **20**, 2229-2247.

MATTIACCI L, DICKE M & POSTHUMUS MA (1995) β-Glucosidase: An elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 2036-2040.

MCCALL PJ, TURLINGS TCJ, LOUGHRIN J, PROVEAUX AT & TUMLINSON JH (1994) Herbivore-induced volatile emissions from cotton (G*ossypium hirsutum* L) seedlings. *Journal of Chemical Ecology*, **20**, 3039-3050.

MCCONN M, CREELMAN RA, BELL E, MULLET JE & BROWSE J (1997) Jasmonate is essential for insect defense *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 5473-5477.

MCKAY SAB, HUNTER WL, GODARD KA, WANG SX, MARTIN DM, BOHLMANN J & PLANT AL (2003) Insect attack and wounding induce traumatic resin duct development and gene expression of (-)-pinene synthase in Sitka spruce. *Plant Physiology*, **133**, 368-378.

MENKE FLH, CHAMPION A, KIJNE JW & MEMELINK J (1999) A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene *Str* interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor, ORCA2. *EMBO Journal*, **18**, 4455-4463.

MEYER CR & MEYER VG (1961) Origin and inheritance in nectariless cotton. *Crop science*, **1**, 167-170.

MICHAUD JP (1990) Observations on nectar secretion in fireweed, *Epilobium angustifolium* L (Onagraceae). *Journal of Apicultural Research*, **29**, 132-137.

MILLER B, MADILAO LL, RALPH S & BOHLMANN J (2005) Insect-induced conifer defense. White pine weevil and methyl jasmonate induce traumatic resinosis, *de novo* formed volatile emissions, and accumulation of terpenoid synthase and putative octadecanoid pathway transcripts in Sitka spruce. *Plant Physiology*, **137**, 369-382.

MITCHELL-OLDS T, SIEMENS D & PEDERSEN D (1996) Physiology and costs of resistance to herbivory and disease in Brassica. *Entomologia Experimentalis et Applicata*, **80**, 231-237.

MITHÖFER A, WANNER G & BOLAND W (2005) Effects of feeding *Spodoptera littoralis* on Lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiology*, **137**, 1160-1168.

MODY K & LINSENMAIR KE (2004) Plant-attracted ants affect arthropod community structure but not necessarily herbivory. *Ecological Entomology*, **29**, 217-225.

MONDOR EB & ADDICOTT JF (2003) Conspicuous extra-floral nectaries are inducible in *Vicia faba*. *Ecology Letters*, **6**, 495-497.

MONTEITH LG (1960) Influence of plants other than the food plants of their host on host-finding by tachinid parasites. *Canadian Entomologist*, **92**, 641-652.

MONTOYA T, NOMURA T, FARRAR K, KANETA T, YOKOTA T & BISHOP GJ

(2002) Cloning the tomato *curl3* gene highlights the putative dual role of the leucine-rich repeat receptor kinase tBRI1/SR160 in plant steroid hormone and peptide hormone signaling. *Plant Cell*, **14**, 3163-3176.

MORAN PJ & THOMPSON GA (2001) Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. *Plant Physiology*, **125**, 1074-1085.

MUMM R, SCHRANK K, WEGENER R, SCHULZ S & HILKER M (2003) Chemical analysis of volatiles emitted from *Pinus sylvestris* after induction by insect oviposition. *Journal of Chemical Ecology*, **29**, 1235-1252.

MUSSER RO, HUM-MUSSER SM, EICHENSEER H, PEIFFER M, ERVIN G, MURPHY JB & FELTON GW (2002) Herbivory: Caterpillar saliva beats plant defences - A new weapon emerges in the evolutionary arms race between plants and herbivores. *Nature*, **416**, 599-600.

NARVÁEZ-VÁSQUEZ J, FLORIN-CHRISTENSEN J & RYAN CA (1999) Positional specificity of a phospholipase A activity induced by wounding, systemin, and oligosaccharide elicitors in tomato leaves. *Plant Cell*, **11**, 2249-2260.

NESS JH (2003) *Catalpa bignonioides* alters extrafloral nectar production after herbivory and attracts ant bodyguards. *Oecologia*, **134**, 210-218.

NEVEU N, GRANDGIRARD J, NENON JP & CORTESERO AM (2002) Systemic release of herbivore-induced plant volatiles by turnips infested by concealed root-feeding larvae *Delia radicum* L. *Journal of Chemical Ecology*, **28**, 1717-1732.

NISHIDA R (2002) Sequestration of defensive substances from plants by Lepidoptera. *Annual Review of Entomology*, **47**, 57-92.

NOYES JS (2003) Universal chalcidoidea database. world wide web electronic publication. ww.nhm.ac.uk/entomology/chalcidoids/index.html [accessed 05-Sep-2003].

O'DONNELL PJ, CALVERT C, ATZORN R, WASTERNACK C, LEYSER HMO & BOWLES DJ (1996) Ethylene as a signal mediating the wound response of tomato plants. *Science*, **274**, 1914-1917.

O'DOWD DJ (1979) Foliar nectar production and ant activity on a neotropical tree, *Ochroma pyramidale*. *Oecologia*, **43**, 233-248.

O'DOWD DJ & CATCHPOLE EA (1983) Ants and extrafloral nectaries: No evidence for plant protection in *Helichrysum* spp-ant interactions. *Oecologia*, **59**, 191-200.

OHME-TAKAGI M & SHINSHI H (1995) Ethylene-inducible DNA-binding proteins that interact with an ethylene-responsive element. *Plant Cell*, **7**, 173-182.

OHME-TAKAGI M, SUZUKI K & SHINSHI H (2000) Regulation of ethylene-induced transcription of defense genes. *Plant and Cell Physiology*, **41**, 1187-1192.

OLIVEIRA PS & FREITAS AVL (2004) Ant-plant-herbivore interactions in the neotropical cerrado savanna. *Naturwissenschaften*, **91**, 557-570.

OLIVEIRA PS, RICO-GRAY V, DIAZ-CASTELAZO C & CASTILLO-GUEVARA C (1999) Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: Herbivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (Cactaceae). *Functional Ecology*, **13**, 623-631.

OROZCO-CÁRDENAS ML, MCGURL B & RYAN CA (1993) Expression of an antisense prosystemin gene in tomato plants reduces resistance toward *Manduca sexta* larvae. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 8273-8276.

OROZCO-CÁRDENAS ML, NARVÁEZ-VÁSQUEZ J & RYAN CA (2001) Hydrogen peroxide acts as a second messen-

ger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *Plant Cell*, **13**, 179-191.

OZAWA R, ARIMURA G, TAKABAYASHI J, SHIMODA T & NISHIOKA T (2000a) Involvement of jasmonate- and salicylate-related signaling pathways for the production of specific herbivore-induced volatiles in plants. *Plant and Cell Physiology*, **41**, 391-398.

OZAWA R, SHIMODA T, KAWAGUCHI M, ARIMURA G, HORIUCHI J, NISHIOKA T & TAKABAYASHI J (2000b) *Lotus japonicus* infested with herbivorous mites emits volatile compounds that attract predatory mites. *Journal of Plant Research*, **113**, 427-433.

PACINI E, NEPI M & VESPRINI JL (2003) Nectar biodiversity: A short review. *Plant Systematics and Evolution*, **238**, 7-21.

PAPAJ DR & LEWIS AC (1993) *Insect learning: ecological and evolutionary perspectives*. Chapman & Hall, New York.

PARÉ PW & TUMLINSON JH (1997) *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiology*, **114**, 1161-1167.

PARÉ PW & TUMLINSON JH (1999) Plant volatiles as a defense against insect herbivores. *Plant Physiology*, **121**, 325-331.

PASCAL L & BELIN-DEPOUX M (1991) On the biological rhythm correlation of plant-ant association - The case of extrafloral nectaries of American Malpighiaceae. *Comptes rendus de l'Académie des sciences. Serie III, Sciences de la vie*, **312**, 49-54.

PASCHOLD A, HALITSCHKE R & BALDWIN IT (2005) Using "mute" plants to translate volatile signals. *Plant Journal*, in press.

PATTENDEN G & WEEDON BLL (1968) Carotenoids and related compounds. part XVIII. Synthesis of *cis*-and di-*cis*-polyenes by reactions of the Wittig type. *Journal of the Chemical Society C-Organic*, **16**, 1984-1997.

PEARCE G & RYAN CA (2003) Systemic signaling in tomato plants for defense against herbivores - Isolation and characterization of three novel defense-signaling glycopeptide hormones coded in a single precursor gene. *Journal of Biological Chemistry*, **278**, 30044-30050.

PEARCE G, STRYDOM D, JOHNSON S & RYAN CA (1991) A polypeptide from tomato leaves induces wound-inducible proteinase-inhibitor proteins. *Science*, **253**, 895-898.

PEARCE G, MOURA DS, STRATMANN J & RYAN CA (2001a) Production of multiple plant hormones from a single polyprotein precursor. *Nature*, **411**, 817-820.

PEARCE G, MOURA DS, STRATMANN J & RYAN CA (2001b) RALF, a 5-kDa ubiquitous polypeptide in plants, arrests root growth and development. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 12843-12847.

PEMBERTON RW & LEE JH (1996) The influence of extrafloral nectaries on parasitism of an insect herbivore. *American Journal of Botany*, **83**, 1187-1194.

PENG JY, LI ZH, XIANG H, HUANG JH, JIA SH, MIAO XX & HUANG YP (2005) Preliminary studies on differential defense responses induced during plant communication. *Cell Research*, **15**, 187-192.

PETITT FL, TURLINGS TCJ & WOLF SP (1992) Adult experience modifies attraction of the leafminer parasitoid *Opius dissitus* (Hymenoptera: Braconidae) to volatile semiochemicals. *Journal of Insect Behavior*, **5**, 623-634.

PICHERSKY E & GERSHENZON J (2002) The formation and function of plant volatiles: Perfumes for pollinator attraction and defense. *Current Opinion in Plant Biology*, **5**, 237-243.

PICKETT JA, RASMUSSEN HB, WOODCOCK CM, MATTHES W & NAPIER JA (2003) Plant stress signalling: Understanding and exploiting plant-plant interactions. *Biochemical Society Transactions*, **31**, 123-127.

POHNERT G, JUNG V, HAUKIOJA E, LEMPA K & BOLAND W (1999) New fatty acid amides from regurgitant of lepidopteran (Noctuidae, Geometridae) caterpillars. *Tetrahedron*, **55**, 11275-11280.

PRESTON CA, LAUE G & BALDWIN IT (2001) Methyl jasmonate is blowing in the wind, but can it act as a plant-plant airborne signal? *Biochemical Systematics and Ecology*, **29**, 1007-1023.

PRICE PW, BOUTON CE, GROSS P, MCPHERON BA, THOMPSON JN & WEIS AE (1980) Interactions among 3 trophic levels - Influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41-65.

PYKE GH (1991) What does it cost a plant to produce floral nectar? *Nature*, **350**, 58-59.

QU N, SCHITTKO U & BALDWIN IT (2004) Consistency of *Nicotiana attenuata*'s herbivore- and jasmonate-induced transcriptional responses in the allotetraploid species *Nicotiana quadrivalvis* and *Nicotiana clevelandii*. *Plant Physiology*, **135**, 539-548.

RAGUSO RA (2004) Why are some floral nectars scented? *Ecology*, **85**, 1486-1494.

RAINE NE, WILLMER P & STONE GN (2002) Spatial structuring and floral avoidance behavior prevent ant-pollinator conflict in a Mexican ant-acacia. *Ecology*, **83**, 3086-3096.

RASHBROOK VK, COMPTON SG & LAWTON JH (1992) Ant-herbivore interactions: Reasons for the absence of benefits to a fern with foliar nectaries. *Ecology*, **73**, 2167-2174.

REGAL PJ (1977) Ecology and evolution of flowering plant dominance. *Science*, **196**, 622-629.

RETTENMEYER CW (1961) Observations on the biology and taxonomy of flies found over swarm raids of army ants (Diptera: Tachinidae, Conopidae). *University of Kansas Science Bulletin*, **42**, 993-1066.

REYMOND P & FARMER EE (1998) Jasmonate and salicylate as global signals for defense gene expression. *Current Opinion in Plant Biology*, **1**, 404-411.

REYMOND P, WEBER H, DAMOND M & FARMER EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell*, **12**, 707-719.

REYMOND P, BODENHAUSEN N, VAN POECKE RMP, KRISHNAMURTHY V, DICKE M & FARMER EE (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell*, **16**, 3132-3147.

RHOADES DF (1983) Responses of alder and willow to attack by tent caterpillars and webworms - Evidence for pheromonal sensitivity of willows. Pages 55-68 in HEDIN PA (ed.) *Plant resistance to insects*. American Chemical Society, Washington DC.

RHYNE CL (1965) Inheritance of extrafloral nectaries in cotton. *Advancing Frontiers in Plant Science*, **13**, 121-135.

RICKSON FR (1971) Glycogen plastids in Müllerian body cells of *Cecropia peltata* - a higher green plant. *Science*, **173**, 344-347.

RICO-GRAY V (1993) Use of plant-derived food resources by ants in the dry tropical lowlands of coastal Veracruz, Mexico. *Biotropica*, **25**, 301-315.

RODRÍGUEZ-CONCEPCIÓN M & BORONAT A (2002) Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A

metabolic milestone achieved through genomics. *Plant Physiology*, **130**, 1079-1089.

RODRIGUEZ-REINOSO F (1998) The role of carbon materials in heterogeneous catalysis. *Carbon*, **36**, 159-175.

ROJO E, SOLANO R & SÁNCHEZ-SERRANO JJ (2003) Interactions between signaling compounds involved in plant defense. *Journal of Plant Growth Regulation*, **22**, 82-98.

RÖSE USR & TUMLINSON JH (2004) Volatiles released from cotton plants in response to *Helicoverpa zea* feeding damage on cotton flower buds. *Planta*, **218**, 824-832.

RÖTTGER U (1979) Untersuchungen zur Wirtswahl der Rübenfliege *Pegomya betae* (Diptera: Anthomyidae). I. Olfaktorische Orientierung zur Wirtspflanze. *Zeitschrift für Angewandte Entomologie*, **87**, 337-348.

ROYO J, LEÓN J, VANCANNEYT G, ALBAR JP, ROSAHL S, ORTEGO F, CASTANERA P & SÁNCHEZ-SERRANO JJ (1999) Antisense-mediated depletion of a potato lipoxygenase reduces wound induction of proteinase inhibitors and increases weight gain of insect pests. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 1146-1151.

RUDGERS JA (2004) Enemies of herbivores can shape plant traits: Selection in a facultative ant-plant mutualism. *Ecology*, **85**, 192-205.

RUDGERS JA, STRAUSS SY & WENDEL JE (2004) Trade-offs among anti-herbivore resistance traits: Insights from Gossypieae (Malvaceae). *American Journal of Botany*, **91**, 871-880.

RUFFNER GA & CLARK WD (1986) Extrafloral nectar of *Ferocactus acanthodes* (Cactaceae): Composition and its importance to ants. *American Journal of Botany*, **73**, 185-189.

RUHREN S & HANDEL SN (1999) Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, **119**, 227-230.

RUTHER J & KLEIER S (2005) Plant-plant signaling: Ethylene synergizes volatile emission in *Zea mays* induced by exposure to (*Z*)-3-hexen-1-ol. *Journal of Chemical Ecology*, **31**, 2217-2222.

RUTHER J, REINECKE A & HILKER M (2002) Plant volatiles in the sexual communication of *Melolontha hippocastani*: Response towards time-dependent bouquets and novel function of (*Z*)-3-hexen-1-ol as a sexual kairomone. *Ecological Entomology*, **27**, 76-83.

RYALS JA, NEUENSCHWANDER UH, WILLITS MG, MOLINA A, STEINER HY & HUNT MD (1996) Systemic acquired resistance. *Plant Cell*, **8**, 1809-1819.

SABELIS MW & VAN DE BAAN HE (1983) Location of distant spider mite colonies by phytoseiid predators: Demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. *Entomologia Experimentalis et Applicata*, **33**, 303-314.

SABELIS MW & DE JONG MCM (1988) Should all plants recruit bodyguards? Conditions for a polymorphic ESS of synomone production in plants. *Oikos*, **53**, 247-252.

SALGADO AG, P. G & DEBOUCK DG (1995) Evidence for two gene pools of the Lima bean, *Phaseolus lunatus* L, in the Americas. *Genetic Resources and crop Evolution*, **42**, 15-28.

SCHAEFER M, BOHLKEN H, REICHSTEIN H, SATTLER K, SCHMIDT E, SICK F & TISCHLER W (1994) *Brohmer - Fauna von Deutschland*. Quelle und Meyer Bestimmungsbücher, Heidelberg.

SCHEER JM & RYAN CA (2002) The systemin receptor SR160 from *Lycopersicon peruvianum* is a member of the LRR receptor kinase family. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 9585-9590.

SCHILMAN PE & ROCES F (2003) Assessment of nectar flow rate and memory for patch quality in the ant *Camponotus rufipes*. *Animal Behaviour*, **66**, 687-693.

SCHITTKO U, PRESTON CA & BALDWIN IT (2000) Eating the evidence? *Manduca sexta* larvae can not disrupt specific jasmonate induction in *Nicotiana attenuata* by rapid consumption. *Planta*, **210**, 343-346.

SCHMELZ EA, ALBORN HT & TUMLINSON JH (2001) The influence of intact-plant and excised-leaf bioassay designs on volicitin- and jasmonic acid-induced sesquiterpene volatile release in *Zea mays*. *Planta*, **214**, 171-179.

SCHMELZ EA, ALBORN HT, BANCHIO E & TUMLINSON JH (2003) Quantitative relationships between induced jasmonic acid levels and volatile emission in *Zea mays* during *Spodoptera exigua* herbivory. *Planta*, **216**, 665-673.

SCHMIDT DD, KESSLER A, KESSLER D, SCHMIDT S, LIM M, GASE K & BALDWIN IT (2004) *Solanum nigrum*: A model ecological expression system and its tools. *Molecular Ecology*, **13**, 981-995.

SCUTAREANU P, BRUIN J, POSTHUMUS MA & DRUKKER B (2003) Constitutive and herbivore-induced volatiles in pear, alder and hawthorn trees. *Chemoecology*, **13**, 63-74.

SCUTAREANU P, DRUKKER B, BRUIN J, POSTHUMUS MA & SABELIS MW (1997) Volatiles from *Psylla*-infested pear trees and their possible involvement in attraction of anthocorid predators. *Journal of Chemical Ecology*, **23**, 2241-2260.

SÉGUY E (1951) *Atlas des Diptères de France, Belgique, Suisse*. Boubée, Paris.

SEO HS, SONG JT, CHEONG JJ, LEE YH, LEE YW, HWANG I, LEE JS & CHOI YD (2001) Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 4788-4793.

SEO S, SANO H & OHASHI Y (1999) Jasmonate-based wound signal transduction requires activation of WIPK, a tobacco mitogen-activated protein kinase. *Plant Cell*, **11**, 289-298.

SEO S, SETO H, KOSHINO H, YOSHIDA S & OHASHI Y (2003) A diterpene as an endogenous signal for the activation of defense responses to infection with Tobacco mosaic virus and wounding in tobacco. *Plant Cell*, **15**, 863-873.

SEO S, OKAMOTO M, SETO H, ISHIZUKA K, SANO H & OHASHI Y (1995) Tobacco MAP kinase: A possible mediator in wound signal transduction pathways. *Science*, **270**, 1988-1992.

SHARON-ASA L, SHALIT M, FRYDMAN A, BAR E, HOLLAND D, OR E, LAVI U, LEWINSOHN E & EYAL Y (2003) Citrus fruit flavor and aroma biosynthesis: Isolation, functional characterization, and developmental regulation of *Cstps1*, a key gene in the production of the sesquiterpene aroma compound valencene. *Plant Journal*, **36**, 664-674.

SHIMODA T, TAKABAYASHI J, ASHIHARA W & TAKAFUJI A (1997) Response of predatory insect *Scolothrips takahashii* toward herbivore-induced plant volatiles under laboratory and field conditions. *Journal of Chemical Ecology*, **23**, 2033-2048.

SHIMODA T, OZAWA R, ARIMURA G, TAKABAYASHI J & NISHIOKA T (2002) Olfactory responses of two specialist insect predators of spider mites toward plant

volatiles from Lima bean leaves induced by jasmonic acid and/or methyl salicylate. *Applied Entomology and Zoology*, **37**, 535-541.

SHIOJIRI K, TAKABAYASHI J, YANO S & TAKAFUJI A (2000) Flight response of parasitoids toward plant-herbivore complexes: A comparative study of two parasitoid-herbivore systems on cabbage plants. *Applied Entomology and Zoology*, **35**, 87-92.

SHONLE I & BERGELSON J (1995) Interplant communication revisited. *Ecology*, **76**, 2660-2663.

SHULAEV V, SILVERMAN P & RASKIN I (1997) Airborne signalling by methyl salicylate in plant pathogen resistance. *Nature*, **385**, 718-721.

SIVASANKAR S, SHELDRICK B & ROTHSTEIN SJ (2000) Expression of allene oxide synthase determines defense gene activation in tomato. *Plant Physiology*, **122**, 1335-1342.

SKIRI HT, GALIZIA CG & MUSTAPARTA H (2004) Representation of primary plant odorants in the antennal lobe of the moth *Heliothis virescens* using calcium imaging. *Chemical Senses*, **29**, 253-267.

SMITH MR (1947) A generic and subgeneric synopsis of the United-States ants, based on the workers (Hymenoptera, Formicidae). *American Midland Naturalist*, **37**, 521-647.

SOBRINHO TG, SCHOEREDER JH, RODRIGUES LL & COLLEVATTI RG (2002) Ant visitation (Hymenoptera: Formicidae) to extrafloral nectaries increases seed set and seed viability in the tropical weed *Triumfetta semitriloba*. *Sociobiology*, **39**, 353-368.

SOLANO R, STEPANOVA A, CHAO QM & ECKER JR (1998) Nuclear events in ethylene signaling: A transcriptional cascade mediated by ethylene-insensitive3 and ethylene-response-factor-1. *Genes & Development*, **12**, 3703-3714.

SPITELLER D & BOLAND W (2003) *N*-(17-acyloxy-acyl)-glutamines: Novel surfactants from oral secretions of lepidopteran larvae. *Journal of Organic Chemistry*, **68**, 8743-8749.

SPITELLER D, POHNERT G & BOLAND W (2001) Absolute configuration of volicitin, an elicitor of plant volatile biosynthesis from lepidopteran larvae. *Tetrahedron Letters*, **42**, 1483-1485.

SPOEL SH, KOORNNEEF A, CLAESSENS SMC, KORZELIUS JP, VAN PELT JA, MUELLER MJ, BUCHALA AJ, MÉTRAUX JP, BROWN R, KAZAN K, VAN LOON LC, DONG XN & PIETERSE CMJ (2003) NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *Plant Cell*, **15**, 760-770.

STANJEK V, HERHAUS C, RITGEN U, BOLAND W & STÄDLER E (1997) Changes in the leaf surface chemistry of *Apium graveolens* (Apiaceae) stimulated by jasmonic acid and perceived by a specialist insect. *Helvetica Chimica Acta*, **80**, 1408-1420.

STAPEL JO, CORTESERO AM, DEMORAES CM, TUMLINSON JH & LEWIS WJ (1997) Extrafloral nectar, honeydew, and sucrose effects on searching behavior and efficiency of *Microplitis croceipes* (Hymenoptera: Braconidae) in cotton. *Environmental Entomology*, **26**, 617-623.

STASWICK PE & TIRYAKI I (2004) The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. *Plant Cell*, **16**, 2117-2127.

STEIDLE JLM & VAN LOON JJA (2002) Chemoecology of parasitoid and predator oviposition behaviour. Pages 291-317 in HILKER M & MEINERS T (eds.)

Chemoecology of insect eggs and egg deposition. Blackwell, London.

STEPHENSON AG (1982) The role of the extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology*, **63**, 663-669.

STOUT MJ, WORKMAN J & DUFFEY SS (1994) Differential induction of tomato foliar proteins by arthropod herbivores. *Journal of Chemical Ecology*, **20**, 2575-2594.

STOUT MJ, FIDANTSEF AL, DUFFEY SS & BOSTOCK RM (1999) Signal interactions in pathogen and insect attack: Systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. *Physiological and Molecular Plant Pathology*, **54**, 115-130.

STRÄßNER M (1999) *Klimadiagramme der Erde - Teil 2: Asien, Lateinamerika, Afrika, Australien und Ozeanien, Polarländer*. Dortmunder Vertrieb für Bau- und Planungsliteratur, Dortmund.

STRAUSS SY, RUDGERS JA, LAU JA & IRWIN RE (2002) Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution*, **17**, 278-285.

STRONG DR & LARSSON S (1994) Is the evolution of herbivore resistance influenced by parasitoids? Pages 261-276 in HAWKINS BA & SHEEHAN W (eds.) *Parasitoid community ecology*. Oxford University Press, Oxford.

SZEKERES M (2003) Brassinosteroid and systemin: Two hormones perceived by the same receptor. *Trends in Plant Science*, **8**, 102-104.

TAKABAYASHI J & DICKE M (1996) Plant-carnivore mutualism through herbivore-induced carnivore attractants. *Trends in Plant Science*, **1**, 109-113.

TAKABAYASHI J, DICKE M & POSTHUMUS MA (1991) Variation in composition of predator-attracting allelochemicals emitted by herbivore-infested plants: Relative influence of plant and herbivore. *Chemoecology*, **2**, 1-6.

TAKABAYASHI J, DICKE M & POSTHUMUS MA (1994a) Volatile herbivore-induced terpenoids in plant-mite interactions - Variation caused by biotic and abiotic factors. *Journal of Chemical Ecology*, **20**, 1329-1354.

TAKABAYASHI J, TAKAHASHI S, DICKE M & POSTHUMUS MA (1995) Developmental stage of herbivore *Pseudaletia separata* affects production of herbivore-induced synomone by corn plants. *Journal of Chemical Ecology*, **21**, 273-287.

TAKABAYASHI J, DICKE M, TAKAHASHI S, POSTHUMUS MA & VANBEEK TA (1994b) Leaf age affects composition of herbivore-induced synomones and attraction of predatory mites. *Journal of Chemical Ecology*, **20**, 373-386.

TAKAHASHI Y, BERBERICH T, MIYAZAKI A, SEO S, OHASHI Y & KUSANO T (2003) Spermine signalling in tobacco: Activation of mitogen-activated protein kinases by spermine is mediated through mitochondrial dysfunction. *Plant Journal*, **36**, 820-829.

TAMAYO MC, RUFAT M, BRAVO JM & SAN SEGUNDO B (2000) Accumulation of a maize proteinase inhibitor in response to wounding and insect feeding and characterization of its activity toward digestive proteinases of *Spodoptera littoralis* larvae. *Planta*, **211**, 62-71.

TEMPEL AS (1983) Bracken fern (*Pteridium aquilinum*) and nectar-feeding ants: A nonmutualistic interaction. *Ecology*, **64**, 1411-1422.

THALER JS (1999) Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature*, **399**, 686-688.

THALER JS, FIDANTSEF AL, DUFFEY SS & BOSTOCK RM (1999) Trade-offs in plant defense against pathogens and herbi-

vores: A field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology*, **25**, 1597-1609.

THISTLE HW, PETERSON H, ALLWINE G, LAMB B, STRAND T, HOLSTEN EH & SHEA PJ (2004) Surrogate pheromone plumes in three forest trunk spaces: Composite statistics and case studies. *Forest Science*, **50**, 610-625.

THOMA I, LOEFFLER C, SINHA AK, GUPTA M, KRISCHKE M, STEFFAN B, ROITSCH T & MUELLER MJ (2003) Cyclopentenone isoprostanes induced by reactive oxygen species trigger defense gene activation and phytoalexin accumulation in plants. *Plant Journal*, **34**, 363-375.

TILMAN D (1978) Cherries, ants and tent caterpillars - Timing of nectar production in relation to susceptibility of caterpillars to ant predation. *Ecology*, **59**, 686-692.

TOMITA A, MORI S & TAMAI Y (1971) Catalytic behavior of carbon on oxidation of cyclohexene. *Carbon*, **9**, 224-225.

TRELEASE W (1881) The foliar nectar gland of *Populus*. *Botanical Gazette*, **6**, 284-290.

TRUITT CL, WEI HX & PARÉ PW (2004) A plasma membrane protein from *Zea mays* binds with the herbivore elicitor volicitin. *Plant Cell*, **16**, 523-532.

TSCHARNTKE T, THIESSEN S, DOLCH R & BOLAND W (2001) Herbivory, induced resistance, and interplant signal transfer in *Alnus glutinosa*. *Biochemical Systematics and Ecology*, **29**, 1025-1047.

TURLINGS TCJ & TUMLINSON JH (1991) Do parasitoids use herbivore-induced plant chemical defences to locate hosts? *Florida Entomologist*, **74**, 42-50.

TURLINGS TCJ & BENREY B (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience*, **5**, 321-333.

TURLINGS TCJ, TUMLINSON JH & LEWIS WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, **250**, 1251-1253.

TURLINGS TCJ, WÄCKERS FL, VET LEM, LEWIS JM & TUMLINSON JH (1993) Learning of host-finding cues by hymenopterous parasitoids. Pages 51-78 in PAPAJ DR & LEWIS AC (eds.) *Insect learning - Ecology and evolutionary perspectives*. Chapman & Hall, New York.

TURLINGS TCJ, LOUGHRIN JH, MCCALL PJ, RÖSE USR, LEWIS WJ & TUMLINSON JH (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 4169-4174.

TURNER JG, ELLIS C & DEVOTO A (2002) The jasmonate signal pathway. *Plant Cell*, **14**, S153-S164.

ULLOA RM, RAICES M, MACINTOSH GC, MALDONADO S & TELLEZ-INON MT (2002) Jasmonic acid affects plant morphology and calcium-dependent protein kinase expression and activity in *Solanum tuberosum*. *Physiologia Plantarum*, **115**, 417-427.

VAN ALPHEN JJM & JERVIS MA (1996). Foraging behaviour in JERVIS M & KIDD N (eds.) *Insect natural enemies*. Chapman & Hall, London.

VAN DEN BOOM CEM, VAN BEEK TA, POSTHUMUS MA, DE GROOT A & DICKE M (2004) Qualitative and quantitative variation among volatile profiles induced by *Tetranychus urticae* feeding on plants from various families. *Journal of Chemical Ecology*, **30**, 69-89.

VAN DER FITS L & MEMELINK J (2000) ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. *Science*, **289**, 295-297.

VAN DER MEIJDEN E & KLINKHAMER PGL (2000) Conflicting interests of plants and

the natural enemies of herbivores. *Oikos*, **89**, 202-208.

VAN DER PUTTEN WH, VET LEM, HARVEY JA & WÄCKERS FL (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology and Evolution*, **16**, 547-554.

VAN DORT HM, JAGERS PP, TERHEIDE R & VANDERWEERDT AJA (1993) *Narcissus trevithian* and *Narcissus geranium* - Analysis and synthesis of compounds. *Journal of Agricultural and Food Chemistry*, **41**, 2063-2075.

VAN LOON JJA, DE BOER JG & DICKE M (2000) Parasitoid-plant mutualism: Parasitoid attack of herbivore increases plant reproduction. *Entomologia Experimentalis et Applicata*, **97**, 219-227.

VAN POECKE RMP & DICKE M (2004) Indirect defence of plants against herbivores: Using *Arabidopsis thaliana* as a model plant. *Plant Biology*, **6**, 387-401.

VAN POECKE RMP, POSTHUMUS MA & DICKE M (2001) Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: Chemical, behavioral, and gene-expression analysis. *Journal of Chemical Ecology*, **27**, 1911-1928.

VAN RIJN PCJ & TANIGOSHI LK (1999) The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. *Experimental & Applied Acarology*, **23**, 281-296.

VAN TOL R, VAN DER SOMMEN ATC, BOFF MIC, VAN BEZOOIJEN J, SABELIS MW & SMITS PH (2001) Plants protect their roots by alerting the enemies of grubs. *Ecology Letters*, **4**, 292-294.

VANCANNEYT G, SANZ C, FARMAKI T, PANEQUE M, ORTEGO F, CASTANERA P & SÁNCHEZ-SERRANO JJ (2001) Hydroperoxide lyase depletion in transgenic potato plants leads to an increase in aphid performance. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 8139-8144.

VANDER MEER R, BREED M, ESPELIE K & WINSTON M (1998) *Pheromone communication in social insects: Ants, wasps, bees, and termites*. Westview Press, Boulder.

VET LEM & DICKE M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, **37**, 141-172.

VET LEM, LEWIS WJ & CARDÉ RT (1995) Parasitoid foraging and learning. Pages 65-101 in CARDÉ R & BELL WJ (eds.) *Chemical ecology of insects. Vol. 2.* Chapman & Hall, New York.

VIJAYAN P, SHOCKEY J, LEVESQUE CA, COOK RJ & BROWSE J (1998) A role for jasmonate in pathogen defense of *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 7209-7214.

VISSER JH (1986) Host odor perception in phytophagous insects. *Annual Review of Entomology*, **31**, 121-144.

VISSER JH & AVÉ DA (1978) General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata*. *Entomologia Experimentalis et Applicata*, **24**, 538-549.

VOELCKEL C & BALDWIN IT (2004) Generalist and specialist lepidopteran larvae elicit different transcriptional responses in *Nicotiana attenuata*, which correlate with larval FAC profiles. *Ecology Letters*, **7**, 770-775.

VOELCKEL C, SCHITTKO U & BALDWIN IT (2001) Herbivore-induced ethylene burst reduces fitness costs of jasmonate- and oral secretion-induced defenses in *Nicotiana attenuata*. *Oecologia*, **127**, 274-280.

VÖLKL W & SULLIVAN DJ (2000) Foraging behaviour, host plant and host location in the aphid hyperparasitoid *Euneura auga-*

rus. *Entomologia Experimentalis et Applicata*, **97**, 47-56.

VON DAHL CC & BALDWIN IT (2004) Methyl jasmonate and *cis*-jasmone do not dispose of the herbivore-induced jasmonate burst in *Nicotiana attenuata*. *Physiologia Plantarum*, **120**, 474-481.

VON WETTSTEIN R (1889) Über die Compositen der österreichisch-ungarischen Flora mit zuckerabscheidenden Hüllschuppen. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Classe, Ser. 1*, **97**, 570-589.

VOS M, BERROCAL SM, KARAMAOUNA F, HEMERIK L & VET LEM (2001) Plant-mediated indirect effects and the persistence of parasitoid-herbivore communities. *Ecology Letters*, **4**, 38-45.

WÄCKERS FL & BEZEMER TM (2003) Root herbivory induces an above-ground indirect defence. *Ecology Letters*, **6**, 9-12.

WÄCKERS FL & BONIFAY C (2004) How to be sweet? Extrafloral nectar allocation by *Gossypium hirsutum* fits optimal defense theory predictions. *Ecology*, **85**, 1512-1518.

WÄCKERS FL, ZUBER D, WUNDERLIN R & KELLER F (2001) The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. *Annals of Botany*, **87**, 365-370.

WANG CX, ZIEN CA, AFITLHILE M, WELTI R, HILDEBRAND DF & WANG XM (2000) Involvement of phospholipase D in wound-induced accumulation of jasmonic acid in *Arabidopsis*. *Plant Cell*, **12**, 2237-2246.

WANG KLC, LI H & ECKER JR (2002) Ethylene biosynthesis and signaling networks. *Plant Cell*, **14**, S131-S151.

WANG ZY & HE JX (2004) Brassinosteroid signal transduction - Choices of signals and receptors. *Trends in Plant Science*, **9**, 91-96.

WASTERNACK C & PARTHIER B (1997) Jasmonate-signalled plant gene expression. *Trends in Plant Science*, **2**, 302-307.

WEISSBECKER B, VAN LOON JJA, POSTHUMUS MA, BOUWMEESTER HJ & DICKE M (2000) Identification of volatile potato sesquiterpenoids and their olfactory detection by the two-spotted stinkbug *Perillus bioculatus*. *Journal of Chemical Ecology*, **26**, 1433-1445.

WILDON DC, THAIN JF, MINCHIN PEH, GUBB IR, REILLY AJ, SKIPPER YD, DOHERTY HM, O'DONNELL PJ & BOWLES DJ (1992) Electrical signaling and systemic proteinase-inhibitor induction in the wounded plant. *Nature*, **360**, 62-65.

WINZ RA & BALDWIN IT (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. IV. Insect-induced ethylene reduces jasmonate-induced nicotine accumulation by regulating putrescine *N*-methyltransferase transcripts. *Plant Physiology*, **125**, 2189-2202.

XU Y, CHANG PFL, LIU D, NARASIMHAN ML, RAGHOTHAMA KG, HASEGAWA PM & BRESSAN RA (1994) Plant defense genes are synergistically induced by ethylene and methyl jasmonate. *Plant Cell*, **6**, 1077-1085.

XU YH, WANG JW, WANG S, WANG JY & CHEN XY (2004) Characterization of GaWRKY1, a cotton transcription factor that regulates the sesquiterpene synthase gene (+)-δ-cadinene synthase-A. *Plant Physiology*, **135**, 507-515.

YAMAKAWA H, KAMADA H, SATOH M & OHASHI Y (1998) Spermine is a salicylate-independent endogenous inducer for both tobacco acidic pathogenesis-related proteins and resistance against tobacco mosaic virus infection. *Plant Physiology*, **118**, 1213-1222.

YAMAKAWA H, KATOU S, SEO S, MITSUHARA I, KAMADA H & OHASHI Y (2004) Plant MAPK phosphatase interacts with calmodulins. *Journal of Biological Chemistry*, **279**, 928-936.

YANG K & JOHNSON MA (1977) Novel carbon catalysis - Oxidation in basic solution. *Journal of Organic Chemistry*, **42**, 3754-3754.

ZACHARIA R (2004). *Desorption of gases from graphitic and porous carbon surfaces*. PhD thesis, Fachbereich Biologie, Chemie, Pharmazie, Berlin.

ZANGERL AR (2003) Evolution of induced plant responses to herbivores. *Basic and Applied Ecology*, **4**, 91-103.

ZHANG SQ & KLESSIG DF (1998) The tobacco wounding-activated mitogen-activated protein kinase is encoded by *SIPK*. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 7225-7230.

ZHU ZH, FINNERTY J, LU GQ & YANG RT (2002) A comparative study of carbon gasification with O_2 and CO_2 by density functional theory calculations. *Energy & Fuels*, **16**, 1359-1368.

ZIMMERMANN JG (1932) Über die extrafloralen Nektarien der Angiospermen. *Botanisches Zentrallblatt Beiheft*, **49**, 99-196.

ZIMMERMANN S, EHRHARDT T, PLESCH G & MÜLLER-RÖBER B (1999) Ion channels in plant signaling. *Cellular and Molecular Life Sciences*, **55**, 183-203.

ZORRO BI I, MAQUET A & BAUDOIN JP (2005) Mating system of wild *Phaseolus lunatus* L and its relationship to population size. *Heredity*, **94**, 153-158.

12. Selbständigkeitserklärung

Entsprechend der Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität (Nr. III, § 5, Ziff. 3) erkläre ich, dass mir die geltende Promotionsordnung der Fakultät bekannt ist. Die vorliegende Arbeit habe ich selbständig und nur unter Verwendung der angegebenen Hilfsmittel, persönlichen Mitteilungen, Quellen und Literatur angefertigt habe. Personen, die an der experimentellen Durchführung, Auswertung des Datenmaterials oder bei der Verfassung der Manuskripte beteiligt waren, sind am Beginn der Arbeit (2. Thesis outline – List of manuscripts and author's contribution) oder am Ende jedes Manuskriptes (Acknowledgements) angegeben. Ich habe nicht die Hilfe eines Promotionsberaters in Anspruch genommen und Dritte haben weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorliegenden Dissertation stehen. Ich habe die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht. Ferner habe ich nicht versucht, diese Arbeit oder eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung bei einer anderen Hochschule als Dissertation einzureichen.

Jena, den . .2006 ……
Christian Kost

13. Curriculum vitae

Personal data

Name:	Christian Kost
Date of birth:	09.09.1975
Place of birth:	Bad Sobernheim, Germany
Home address:	Franziskastr. 28, 55569 Monzingen, Germany
marital status:	unmarried
E-mail:	Christiankost@web.de, ckost@ice.mpg.de

Scientific career

since 04/2003	PhD student at the Max Planck Institute for Chemical Ecology in Jena and the Friedrich-Schiller-University Jena.
08/01 – 03/03	Research project at the University of Würzburg in the Department of Animal Ecology and Tropical Biology of Prof. K. E. Linsenmair: *The influence of driver ants* (Dorylus (Anomma) nigricans *on soil-living arthropod communities in Côte d'Ivoire, West-Africa*.
07/00 – 07/01	Diploma thesis: *Foraging strategies and diversity of the symbiotic Streptomycete in leaf-cutting ants (Formicidae: Attini)* supervised by Dr. Rainer Wirth (Department of General Botany) and Dr. habil. Matthias Redenbach (Department of Genetics).
10/95 – 07/00	University of Kaiserslautern, Diploma in biology; Major: botany, minors: genetics, ecology and biochemistry

Education

01/87 - 08/95	Emanuel Felke Gymnasium Bad Sobernheim (Abitur)
07/83 - 06/86	Grundschule Monzingen

Practical training and stays abroad

10/04 - 12/04 and 10/03 - 12/03	Field work at sites close to Puerto Escondido, Oaxaca, Mexico.
05/02 - 09/02 and 09/01 - 11/01	Field work in the Comoé National Park, Côte d'Ivoire, West-Africa.
12/99 - 03/00	Field work in Paracou, French Guiana.
07/99 - 09/99	Volunteer on Utila, Honduras.
03/99 - 04/99	Field work in the Sahara, Tunisia.

Publications

ARIMURA GI, **KOST C** & BOLAND W (2005) Herbivore-induced, indirect plant defences. *Biochimica et Biophysica Acta: Lipids and Lipid Metabolism*, **1734**, 91-111.

KOST C, DE OLIVEIRA EG, KNOCH TA & WIRTH R (2005) Spatio-temporal permanence and plasticity of foraging trail systems in young and mature leaf-cutting ant colonies (*Atta* spp). *Journal of Tropical Ecology*, **21**, 1-12.

KOST C & HEIL M (2005) Increased availability of extrafloral nectar reduces herbivory in Lima beans (*Phaseolus lunatus*, Fabaceae). *Basic and Applied Ecology*, **6**, 237-248.

KOST C & HEIL M (2005) Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. *Journal of Ecology*, accepted.

DIETRICH R, PLOSS K, **KOST C** & HEIL M (2005) Pathogen resistance induction accelerates senescence in local and systemic tissues of *Arabidopsis thaliana*. submitted to *European Journal of Plant Pathology.*

LEAL IR, FISCHER A, **KOST C,** TABARELLI M & WIRTH R (2005) Ant-mediated protection against herbivores and nectar thieves in *Passiflora coccinea* flowers. submitted to *Ecoscience.*

MEKEM SONWA M, **KOST C**, BIEDERMANN A, WEGENER R, SCHULZ S & BOLAND W (2005) Dehydrogenation of ocimene by active carbon: Artefact formation during headspace sampling from leaves of *Phaseolus lunatus*. submitted to *Tetrahedron*.

Oral presentations

KOST C, WEISSER W, BOLAND W & HEIL M (2005) Lima bean neighbourhood watch - Herbivore-induced volatiles induce an indirect defence in neighbouring plants. *Kurt-Mothes-Doktoranden-Workshop*, Leibniz Institut für Pflanzenbiochemie, Halle, Germany.

KOST C, BOLAND W & HEIL M (2005) Two induced defences benefit Lima bean in nature. *17. Irseer Naturstofftage der DECHEMA e.V.*, Irsee, Germany.

KOST C & HEIL M (2004) Indirekte Verteidigung der Limabohne im Freiland. *Multitrophische Interaktionen*, Göttingen, Germany.

KOST C (2004) Duft oder Nektar? - Wie sich die Limabohne im Freiland gegen Fraßfeinde verteidigt. *27. Fränkisch-Mitteldeutsches Naturstoffchemiker-Treffen*, Bayreuth, Germany.

KOST C, MODY K & LINSENMAIR KE (2002) Determinants of local biodiversity II: Driver ants. *1st International BIOTA-West Workshop*, Abidjan, Côte d'Ivoire, West-Africa.

KOST C & WIRTH R (2001) Symbiotic soil bacteria as broadband fungicides? High diversity of Streptomycetes in leaf-cutting ant colonies in French Guiana. *Gesellschaft für Tropenökologie*, Bremen, Germany.

Poster presentations

KOST C & HEIL M (2005) Lima bean neighbourhood watch - Herbivore-induced volatiles induce an indirect defence in neighbouring plants. *21st annual meeting of the International Society for Chemical Ecology*, Washington, United States of America.

KOST C, BOLAND W & HEIL M (2005) Two induced defences benefit Lima bean in nature. *17. Irseer Naturstofftage der DECHEMA e.V.*, Irsee, Germany.

KOST C & HEIL M (2004) Two induced indirect defences benefit Lima bean plants in nature. *12th Symposium on Insect-Plant Relationships*, Berlin, Germany.

MODY K, **KOST C** & LINSENMAIR KE (2002) Small-scale mosaics of arthropod communities in natural habitats: patterns and determinants. *1st International BIOTA-West Workshop*, Abidjan, Côte d'Ivoire, West Africa.

MODY K, **KOST C** & LINSENMAIR KE (2001) Determinants of small-scale mosaics of arthropod communities in natural and anthropogenically disturbed habitats. *Annual Status Seminar of BIOLOG*, Bonn, Germany.

WIRTH R, **KOST C** & SCHMIDT S (2001) Costs of trail construction and maintenance may affect the pattern of herbivory in leaf-cutting-ants. *Verhandlungen der Gesellschaft für Ökologie*, Band 31; Basel, Switzerland.

BÖTTCHER IG, **KOST C**, WIRTH R & REDENBACH M (2000) Identification and analysis of actinomycetes derived from fungus growing leaf cutting ants. *Vereinigung für Allgemeine und Angewandte Mikrobiologie: Biologie bakterieller Naturstoffproduzenten*, Bonn, Germany.

Jena, den . .2006 ……
Christian Kost